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CONTENTS

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THE PHILIPPINE
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CONTRIBUTION TO THE SEROLOGIC GROUPING OF BACILLUS DYSENTERIÆ BASED UPON THE QUALITY OF ANTIGEN AND NORMAL AGGLUTININS

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As a diagnostic method the agglutination reaction is one of the oldest immune reactions known. The well-known Gruber-Vidal reaction for the diagnosis of typhoid fever has been used for practical purposes, and the principle on which this method is based was in its time applied to serodiagnosis of other bacterial infections.

The identification of isolated strains (intestinal pathogenic bacteria, in particular) by means of agglutination is the most convenient and generally used laboratory method. With the view of specificity, however, research on agglutinins was mainly devoted to the study of immune agglutinins. The normal, or natural, agglutinins have had a theoretical interest only. Bürgi [1] in 1907 admits that very little research has been done on normal agglutinins. His is the most exhaustive study found in the available literature; it includes normal sera from numerous animals and a long series of various microörganisms—among others, *Bacillus dysenteriæ*.

In the course of systematic study by several members of the staff of the Bureau of Science concerning the bacteriology and immunology of bacillary dysentery it became desirable to secure information as to the agglutination of *B. dysenteriæ* by normal

[1] Archiv. für Hygiene 62 (1907) 239–276.

sera of various animals, possible donors of agglutinant sera. Therefore, the investigation here discussed was carried out. In the course of the investigation concerning the presence or absence of agglutinins toward *B. dysenteriæ* (both the Shiga and the Flexner types) in normal sera of laboratory animals, a constant phenomenon was encountered when the serum of normal rabbits was used. This finding was considered of sufficient interest to warrant further investigation of the phenomenon.

STUDY OF AGGLUTININS IN FRESH AND INACTIVATED SERA OF VARIOUS NORMAL ANIMALS TOWARD BACILLUS DYSENTERIÆ (SHIGA AND FLEXNER TYPES)

TECHNIC

Fresh twenty-four-hour-old cultures grown on acid agar (+ 1 phenolphthalein) of one known strain of *B. dysenteriæ* Shiga and one known strain of *B. dysenteriæ* Flexner were used in these agglutination tests.

Sera of the following animals were used: Guinea pig, rabbit, horse, mule, goat, sheep, and monkey. The sera were obtained by bleeding the animals and allowing the blood to coagulate. The clear supernatant serum was centrifuged and decanted carefully. The clear serum of each animal was divided into two equal parts and one part was kept as fresh serum and the other part was heated for thirty minutes at 56° C. The portion kept as fresh serum was again divided into two parts, one for the agglutination test with Shiga strain and one for the agglutination test with Flexner strain. The inactivated (heated) serum of each animal was also divided into two parts, one for agglutination test with Shiga and one for the test with Flexner. Dilutions of the different sera were then made in the following manner:

Seven rows of small test tubes were set up in a rack, eight tubes to each row and one row for each animal. One cubic centimeter of sterile salt solution (0.9 per cent) was introduced into each tube except the first one. To the first tube and to the second tube of each row 1 cubic centimeter of the serum of the respective animal was added. Thus, the second tube of each row contained 1 cubic centimeter of serum and 1 cubic centimeter of salt solution. The contents of the tube were mixed well and 1 cubic centimeter of the mixture was introduced into the third tube, and so on. From the last tube 1 cubic centi-

meter was discarded. In this way, the first tube of each row contained a serum dilution of 1 : 1; the second, a dilution of 1 : 2; the third, 1 : 4; and so on. Emulsions of fresh twenty-four-hour-old acid agar cultures were prepared; one of *B. dysenteriæ* Shiga and one of *B. dysenteriæ* Flexner. This was done by adding 5 cubic centimeters of sterile salt solution (0.9 per cent) to each culture tube. Several culture tubes of acid agar had been planted, some with *B. dysenteriæ* Shiga and some with *B. dysenteriæ* Flexner. With a platinum loop the growth was well emulsified by shaking the tube to secure a homogenous suspension. Then the tubes were allowed to stand for fifteen minutes, or until the larger particles had settled. The homogenous emulsions were then transferred to sterile test tubes.

With sterile pipettes 1 cubic centimeter of the corresponding emulsion was added to each of the tubes containing the corresponding dilution of sera. The results were read after twenty-four hours and they are tabulated in Table 1. In this table the ratios indicate dilutions of the serum; final dilutions are double these ratios. The culture used was a twenty-four-hour-old acid agar culture, emulsified in salt solution (0.9 per cent).

In this and other tables the following symbols are used:

+ + + +	= Complete.
+ + +	= Almost complete.
+ +	= Partial.
+	= Trace.
±	= Doubtful.
—	= Negative.
B	= Boiling.
RS	= Rabbit serum.
R	= Regular.

TABLE 1.—*Showing results of agglutination tests with one strain of Bacillus dysenteriæ Shiga and one strain of B. dysenteriæ Flexner by fresh and inactivated sera of various normal animals.*

	Shiga (fresh serum).							
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Guinea pig	—	—	—	—	—	—	—	—
Rabbit	—	—	—	—	—	—	—	—
Horse	(*)	++++	++++	++++	++++	+++	+	—
Mule	++++	++++	++++	++++	++++	+++	—	—
Goat	+++	+++	++	+	—	—	—	—
Sheep	++++	++++	++++	++++	++	—	—	—
Monkey	—	—	—	—	—	—	—	—

* Dissolved.

TABLE 1. *Showing results of agglutination tests with one strain of Bacillus dysenteriæ Shiga and one strain of B. dysenteriæ Flexner by fresh and inactivated sera of various normal animals*—Continued.

	Shiga (inactivated serum).							
	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Guinea pig	—	—	—	—	—	—	—	—
Rabbit	—	—	—	—	—	—	—	—
Horse	++++	++++	++++	++++	++++	++	+	—
Mule	++	+++	+++	+++	+++	+	—	—
Goat	+++	+++	++	+	—	—	—	—
Sheep	++++	++++	++++	++++	++	+	—	—
Monkey	—	—	—	—	—	—	—	—
	Flexner (fresh serum).							
Guinea pig	—	—	—	—	—	—	—	—
Rabbit	++	++	++	++	+	—	—	—
Horse	++	++++	++++	++++	++++	++++	++++	+++
Mule	++++	++++	++++	++++	++++	+++	+	—
Goat	++++	++++	++++	+++	++	—	—	—
Sheep	++++	++++	++++	++++	++++	++	+	—
Monkey	—	—	—	—	—	—	—	—
	Flexner (inactivated serum).							
Guinea pig	—	—	—	—	—	—	—	—
Rabbit	++	+	—	—	—	—	—	—
Horse	++++	++++	++++	++++	++++	++++	++	++
Mule	++	+++	+++	+++	+++	+++	++	—
Goat	++++	++++	++++	++++	++	+	—	—
Sheep	++++	++++	++++	++++	++	+	—	—
Monkey	—	—	—	—	—	—	—	—

From the results of these experiments it is evident that—

1. The sera of horse, mule, sheep, and goat contain normal agglutinins for both the Shiga and the Flexner types of *B. dysenteriæ*, the strength of the agglutinant power decreasing in the order mentioned.

2. No normal agglutinins were found to exist in the sera of guinea pig or monkey.

3. The inactivation of the sera of horse, mule, sheep, and goat did not alter appreciably the titer of the sera.

4. The serum of rabbit behaved peculiarly, in as much as it agglutinated the Flexner type of *B. dysenteriæ* but not the Shiga type.

5. On heating, the titer of rabbit serum toward the Flexner type decreased considerably.

The peculiar behavior of the rabbit sera was further investigated. In the first place, it had to be established whether it is a general characteristic of rabbit serum or simply a peculiarity of the particular strains of *B. dysenteriæ* used in these experiments. For that reason all available strains of the Shiga and the Flexner types of *B. dysenteriæ*, partly isolated locally from cases and carriers and partly obtained from abroad, were subjected to quantitative agglutination tests with normal rabbit serum. Furthermore, spontaneous agglutination in physiological salt solution and spontaneous agglutination after boiling for one hour (eventually two hours) were investigated in order to see if and how far the state of the antigen is responsible for the interesting finding. The absorption experiments were performed also with the view to determine the existence of agglutinins. It should be emphasized that sera of thirty-three normal rabbits were used in the course of this investigation and all fundamentally behaved in the same manner.

TECHNIC OF THE QUANTITATIVE AGGLUTINATION TEST WITH NORMAL RABBIT SERUM AND ALL AVAILABLE STRAINS OF BACILLUS DYSENTERIÆ

Transplants were made in acid agar the day previous to the test, of all the Shiga and Flexner strains available. The quantitative agglutination test was done in the following manner:

A certain number of normal rabbits were bled. The blood was obtained from the heart of the rabbit by puncture and placed in sterile test tubes (previously rinsed with salt solution to prevent hæmolysis) and allowed to stand in the incubator for one hour. Then the tubes were put in the ice box until the serum separated completely, and the clear sera were pipetted off, pooled, and centrifuged. The clear supernatant serum was decanted and placed in a sterile tube ready for use.

The agglutination test was carried out in the same manner as described before. (See Table 2.)

STUDY OF QUALITATIVE ANTIGEN ANALYSIS OF THE STRAINS OF BACILLUS DYSENTERIÆ SHIGA AND BACILLUS DYSENTERIÆ FLEXNER

All the available Flexner and Shiga fresh twenty-four-hour-old cultures were emulsified in salt solution (0.9 per cent) by adding 5 cubic centimeters of salt solution to one culture slant. The emulsion of each strain was divided into two parts. One part was boiled for one hour at 100° C.; the other part remained as live culture emulsion. The object was to find out the effect of boiling upon the agglutinability of the emulsions. Results were read next day. (See Table 2.)

TABLE 2.—*Showing the results of agglutination tests using normal rabbit serum and all available strains of Bacillus dysenteriæ (Shiga and Flexner types); the results of spontaneous agglutination upon boiling are also shown.*

Culture No.	Titer, fresh rabbit serum.				
	1:1	1:2	1:4	1:8	1:16
1 Shiga	—	—	—	—	—
2 Shiga	—	—	—	—	—
3 Shiga	—	—	—	—	—
4 Shiga	—	—	—	—	—
5 Shiga	—	—	—	—	—
6 Shiga	—	—	—	—	—
7 Shiga	—	—	—	—	—
8 Shiga	—	—	—	—	—
9 Shiga	—	—	—	—	—
10 Shiga	—	—	—	—	—
11 Shiga	—	—	—	—	—
12 Shiga	—	—	—	—	—
13 Shiga	—	—	—	—	—
15 Shiga	—	—	—	—	—
16 Shiga	—	—	—	—	—
17 Shiga	—	—	—	—	—
18 Shiga	—	—	—	—	—
918–2 Shiga	—	—	—	—	—
125,140 Shiga	—	—	—	—	—
Stock Shiga	—	—	—	—	—
T. Mendiola Shiga	—	—	—	—	—
F. Pili Shiga	—	—	—	—	—
Felix Flexner	++++	++++	++++	++	++
185 Carrier Flexner	++++	++++	+++	—	—
1 Flexner	++++	++++	++++	+++	+
2 Flexner	++++	++++	+++	+	—
3 Flexner	++++	++++	++++	++	—
4 Flexner	++++	++++	+++	++	—
5 Flexner	++++	++++	++	—	—
6 Flexner	++++	++++	+++	++	++
7 Flexner	++++	++++	++++	++	—
8 Flexner[b]	—	++++	—	—	—
9 Flexner	++++	++++	++++	++++	++
10 Flexner	++++	++++	++	++	+
11 Flexner	++++	++++	++++	++++	+
12 Flexner	++++	++++	++++	++++	+++
2 Flexner	++++	++++	+++	—	—
3 Flexner	++++	++++	+	—	—
4 Flexner	++++	++++	+++	++	+
9 Flexner	++++	++++	+++	+	—
10 Flexner	++++	++++	+++	+++	±
11 Flexner	++++	++++	++++	+++	—
12 Flexner	++++	++++	+	+	—
14 Flexner	++++	++++	+++	++	+

[b] Contaminated.

TABLE 2.—*Showing the results of agglutination tests using normal rabbit serum and all available strains of Bacillus dysenteriæ (Shiga and Flexner types); the results of spontaneous agglutination upon boiling are also shown*—Continued.

Culture No.	Titer, fresh rabbit serum.				
	1:1	1:2	1:4	1:8	1:16
15 Flexner	++++	++++	+++	+++	—
16 Flexner	++++	++++	+++	+	±
17 Flexner	++++	++++	++	+	—
18 Flexner	++++	+++	+++	+	—
19 Flexner	++++	+++	+++	++	++
20 Flexner	++++	++++	++++	+	—
21 Flexner	++++	++++	++++	+++	—
22 Flexner	++++	++++	++++	±	—
23 Flexner	++++	++++	++++	+	—
24 Flexner	++++	++++	++++	++	—
25 Flexner	++++	++++	++++	+	+
26 Flexner	++++	++++	++++	++++	++++
39 Flexner	++++	++++	++	±	—
40 Flexner	++++	++++	++++	+++	+++
41 Flexner	++++	++++	+++	+	—
1 Flexner	++++	++++	++	+	—
Pickering Flexner	++++	++++	++++	++++	+++
Stock Flexner	++++	++++	++++	++++	++++
Ordoñez Flexner	++++	++++	++++	+++	—
Baniquid Flexner	++++	++++	++++	++++	++++
Pili Flexner	++++	++++	++++	++++	+++
Marabal Flexner	++++	++++	++++	++	—
D. Garcia Flexner	++++	++++	++++	++++	++++
42 Shiga	+++	+++	+++	+	—
43 Shiga	++++	++++	++++	+++	—
44 Shiga	++++	+++	+++	+	—
56 Shiga	++++	++++	++++	++++	+++
59 Shiga	++++	+++	+++	+++	+++
60 Shiga	++++	++++	++++	+	+
66 Shiga	++++	++++	++++	+++	+
67 Shiga	++++	++++	++++	++++	++++
68 Shiga	++++	++++	++++	++	+
69 Shiga	++++	++++	++++	++++	++++
70 Shiga	++++	++++	++++	++++	++++
71 Shiga	++++	++++	++++	++++	++++
D. Flores Shiga	+[a]	+	+	+	+
M. Suzuki Shiga	++++	+++	+++	++	++
A. Espiritu Shiga	+	+	—	—	—
D. Salvatierra Shiga	++++	+++	++	++	—
J. Icaro Shiga	+?	—	—	—	—
S. Palisoc Shiga	++++	++++	++	—	—
M. Reyes Shiga	+	+	±	—	—

[a] A few large flakes with normal rabbit serum.

TABLE 2.—*Showing the results of agglutination tests using normal rabbit serum and all available strains of Bacillus dysenteriæ (Shiga and Flexner types); the results of spontaneous agglutination upon boiling are also shown*—Continued.

Culture No.	Titer, fresh rabbit serum.					
	1:32		1:64		1:128	
1 Shiga	—		—		—	
2 Shiga	—		—		—	
3 Shiga	—		—		—	
4 Shiga	—		—		—	
5 Shiga	—		—		—	
6 Shiga	—		—		—	
7 Shiga	1:32	1:64	1:128	1:256	1:512	1:1024
8 Shiga	—	—	—	—	—	—
9 Shiga	—	—	—	—	—	—
10 Shiga	—	—	—	—	—	—
11 Shiga	—	—	—	—	—	—
12 Shiga	—	—	—	—	—	—
13 Shiga	—	—	—	—	—	—
15 Shiga	—	—	—	—	—	—
16 Shiga	—	—	—	—	—	—
17 Shiga	—	—	—	—	—	—
18 Shiga	—	—	—	—	—	—
918–2 Shiga	—	—	—	—	—	—
125,140 Shiga	—	—	—	—	—	—
Stock Shiga	—	—	—	—	—	—
T. Mendiola Shiga	—	—	—	—	—	—
F. Pili Shiga	—	—	—	—	—	—
Felix Flexner	+	+	±	—	—	—
185 Carrier Flexner	—	—	—	—	—	—
1 Flexner	+	—	—	—	—	—
2 Flexner	—	—	—	—	—	—
3 Flexner	—	—	—	—	—	—
4 Flexner	—	—	—	—	—	—
5 Flexner	—	—	—	—	—	—
6 Flexner	—	—	—	—	—	—
7 Flexner	—	—	—	—	—	—
8 Flexner[b]	—	—	—	—	—	—
9 Flexner	—	—	—	—	—	—
10 Flexner	—	—	—	—	—	—
11 Flexner	—	—	—	—	—	—
12 Flexner	+	—	—	—	—	—
2 Flexner	—	—	—	—	—	—
3 Flexner	—	—	—	—	—	—
4 Flexner	±	—	—	—	—	—
9 Flexner	—	—	—	—	—	—
10 Flexner	—	—	—	—	—	—
11 Flexner	—	—	—	—	—	—
12 Flexner	—	—	—	—	—	—
14 Flexner	—	—	—	—	—	—

[b] Contaminated.

TABLE 2.—*Showing the results of agglutination tests using normal rabbit serum and all available strains of Bacillus dysenteriæ (Shiga and Flexner types); the results of spontaneous agglutination upon boiling are also shown*—Continued.

Culture No.	Titer, fresh rabbit serum.					
	1:32		1:64		1:128	
15 Flexner	—	—	—	—	—	—
16 Flexner	—	—	—	—	—	—
17 Flexner	—	—	—	—	—	—
18 Flexner	—	—	—	—	—	—
19 Flexner	—	—	—	—	—	—
20 Flexner	—	—	—	—	—	—
21 Flexner	—	—	—	—	—	—
22 Flexner	—	—	—	—	—	—
23 Flexner	—	—	—	—	—	—
24 Flexner	—	—	—	—	—	—
25 Flexner	—	—	—	—	—	—
26 Flexner	++	+	—	—	—	—
39 Flexner	—	—	—	—	—	—
40 Flexner	—	—	—	—	—	—
41 Flexner	—	—	—	—	—	—
1 Flexner	—	—	—	—	—	—
Pickering Flexner	++	+	—	—	—	—
Stock Flexner	++++	++++	+++	++	—	—
Ordoñez Flexner	—	—	—	—	—	—
Baniquid Flexner	+++	+	—	—	—	—
Pili Flexner	+++	+	±	—	—	—
Marabal Flexner	—	—	—	—	—	—
D. García Flexner	+++	++	—	—	—	—
42 Shiga	—	—	—	—	—	—
43 Shiga	—	—	—	—	—	—
44 Shiga	—	—	—	—	—	—
56 Shiga	+	—	—	—	—	—
59 Shiga	+	+	—	—	—	—
60 Shiga	—	—	—	—	—	—
66 Shiga	—	—	—	—	—	—
67 Shiga	++++	++++	+	—	—	—
68 Shiga	—	—	—	—	—	—
69 Shiga	++++	++++	+	—	—	—
70 Shiga	++++	++++	++++	—	—	—
71 Shiga	++++	++++	++++	++++	++++	++++
D. Flores Shiga	+	+	+	+	—	—[a]
M. Suzuki Shiga	+	—	—	—	—	—
A. Espiritu Shiga	—	—	—	—	—	—
D. Salvatierra Shiga	—	—	—	—	—	—
J. Icaro Shiga	—	—	—	—	—	—
S. Palisoc Shiga	—	—	—	—	—	—
M. Reyes Shiga	—	—	—	—	—	—

[a] Two hours.

TABLE 2.—*Showing the results of agglutination tests using normal rabbit serum and all available strains of Bacillus dysenteriæ (Shiga and Flexner types); the results of spontaneous agglutination upon boiling are also shown*—Continued.

Culture No.	100 °C. agglutination one hour.	Spontaneous agglutination.	Reaction in mannite.	Source.
1 Shiga	—	—	—	Alabang.
2 Shiga	—	—	—	Do.
3 Shiga	—	—	—	Do.
4 Shiga	—	—	—	Do.
5 Shiga	—	—	—	Do.
6 Shiga	—	—	—	Do.
7 Shiga	—	—	—	Do.
8 Shiga	—	—	—	Do.
9 Shiga	—	—	—	Do.
10 Shiga	—	—	—	Do.
11 Shiga	—	—	—	Do.
12 Shiga	—	—	—	Do.
13 Shiga	—	—	—	Do.
15 Shiga	—	—	—	Do.
16 Shiga	—	—	—	Do.
17 Shiga	—	—	—	Do.
18 Shiga	—	—	—	Do.
918 2 Shiga	—	—	—	P. I., Dr. Vazquez.
125,140 Shiga	—	—	—	P. I., Dr. Lacy.
Stock Shiga	—	—	—	Bureau of Science Laboratory.
T. Mendiola Shiga	—	—	—	Carrier, Dr. Vazquez.
F. Pili Shiga	—	—	—	Do.
Felix Flexner	++++[a]	—	+	Do.
185 Carrier Flexner	—	—	+	Do.
1 Flexner	—	—	+	Alabang.
2 Flexner	—	—	+	Do.
3 Flexner	—	—	+	Do.
4 Flexner	—	—	+	Do.
5 Flexner	—	—	+	Do.
6 Flexner	—	—	+	Do.
7 Flexner	—	—	+	Do.
8 Flexner[b]	—	—	+	Do.
9 Flexner	—	—	+	Do.
10 Flexner	—	—	+	Do.
11 Flexner	—	—	+	Do.
12 Flexner	—	—	+	Do.
2 Flexner	—	—	+	Morishima.
3 Flexner	—	—	+	Do.
4 Flexner	—	—	+	Do.
9 Flexner	—	—	+	Do.
10 Flexner	—	—	+	Do.
11 Flexner	—	—	+	Do.
12 Flexner	—	—	+	Do.
14 Flexner	—	—	+	Do.
15 Flexner	—	—	+	Do.

[a] Large flakes.

[b] Contaminated.

TABLE 2.—*Showing the results of agglutination tests using normal rabbit serum and all available strains of Bacillus dysenteriæ (Shiga and Flexner types); the results of spontaneous agglutination upon boiling are also shown*—Continued.

Culture No.	100 °C. agglutination one hour.	Spontaneous agglutination.	Reaction in mannite.	Source.
16 Flexner	—	—	+	Morishima.
17 Flexner	—	—	+	Do.
18 Flexner	—	—	+	Do.
19 Flexner	—	—	+	Do.
20 Flexner	—	—	+	Do.
21 Flexner	—	—	+	Do.
22 Flexner	—	—	+	Do.
23 Flexner	—	—	+	Do
24 Flexner	—	—	+	Do.
25 Flexner	—	—	+	Do.
26 Flexner	—	—	+	Do.
39 Flexner	—	—	+	Do.
40 Flexner	—	—	+	Do.
41 Flexner	—	—	+	Do.
1 Flexner	++++	—	+	Do.
Pickering Flexner	++++	—	+	Mr. Pickering.
Stock Flexner	—	—	+	Bureau of Science Laboratory.
Ordoñez Flexner	—	—	+	Carrier, Dr. Vazquez.
Baniquid Flexner	++++[a]	—	+	Do.
Pili Flexner	—	—	+	Do.
Marabal Flexner	—	—	+	Do.
D. Garcia Flexner	—	—	+	Do.
42 Shiga	— —[c]	—	—	P. I., Calhoun.
43 Shiga	+?	—	—	P. I., Bellones.
44 Shiga	++	—	—	P. I., Autopsy 10028.
56 Shiga	++++	—	—	P. I., old Laboratory, Dr. Vazquez.
59 Shiga	+?	—	—	P. I., Laboratory, strain Kusama.
60 Shiga	++++	—	—	P. I., Leyte.
66 Shiga	++	—	—	Autopsy 168.
67 Shiga	++++	—	—	A. M. school, U. S.
68 Shiga	+	—	—	Hygiene Lab., U. S.
69 Shiga	++	—	—	Rockefeller Inst., U. S.
70 Shiga	++++	—	—	Hygiene Laboratory, U. S.
71 Shiga	++++	++++	—	Do.
D. Flores Shiga	— —[c]	—	—	P. G. H., Dr. Garcia.
M. Suzuki Shiga	—	—	—	Carrier, Dr. Vazquez.
A. Espiritu Shiga	—	—	—	Do.
D. Salvatierra Shiga	—	—	—	Do.
J. Icaro Shiga	—	—	—	Do.
S. Palisoc Shiga	—	—	—	Do.
M. Reyes Shiga	++++[a]	—	—	Do.

[a] Large flakes.

[c] Two hours.

The results of these tests, as compiled in Table 2, brought out the interesting fact that all of the available strains of *B. dysenteriæ* (Flexner type) were readily agglutinable by normal rabbit serum, but only one of them showed agglutination upon boiling for one hour. In the series of *B. dysenteriæ* (Shiga type) more than half of the strains were plainly inagglutinable by rabbit serum. These strains showed no agglutination upon boiling for one hour. As to the agglutinable group of Shiga strains it must be stated that, even though the agglutination was complete at times, in as much as the supernatant fluid in the completely agglutinated tubes was clear, the sediment was found to be rather loose and not so firmly packed as in the case of the Flexner type. The agglutinable group of the Shiga strains falls again into two subdivisions: one, the larger, the members of which agglutinate spontaneously on boiling; and the other, smaller, the members of which do not agglutinate on boiling. The great majority of the strains belonging in the last subdivision are, interestingly enough, strains isolated from carriers. Furthermore, there appears to be a certain quantitative relation between the serum agglutination and the spontaneous agglutination, provoked by boiling for one hour. One spontaneously agglutinable Shiga strain was included in the collection.

STUDY OF THE ADSORPTION OF AGGLUTININS FROM NORMAL RABBIT SERUM BY BACILLUS DYSENTERIÆ, SHIGA AND FLEXNER TYPES

The pooled sera of two rabbits were diluted with an equal amount of sterile salt solution (0.9 per cent) and divided into three equal parts, and each part was placed in a sterile centrifuge tube. The first tube was marked "Shiga;" the second, "Flexner;" and the third, "Control."

Transplants of the cultures to be used were made the day before. These fresh twenty-four-hour-old cultures (four slants of each) were used in the adsorption test. One strain of Flexner type and one strain of Shiga type, both agglutinable by rabbit normal serum, were used. The tube marked "Control" received nothing but the serum and the salt solution. The three tubes were placed in the incubator for one hour. Then they were taken out and placed on top of the incubator, and left there until the next day.

The tube containing serum treated with the Flexner strain was found completely agglutinated the following day. The serum in the tube in which the Shiga strain was emulsified was still somewhat turbid. The control tube remained clear.

The test tubes containing serum and bacterial emulsion were centrifuged until clear. The clear supernatant serum in each tube was carefully decanted and transferred to two other sterilized centrifuge tubes which were marked "Flexner" and "Shiga," respectively. The same procedure of adsorption was repeated a second time, with fresh cultures of the same strains.

A certain amount of agglutination was noticed next day in the tube marked "Flexner," but the supernatant liquid was very turbid. No agglutination was noticed in the tube marked "Shiga." The control serum was clear. The tubes were centrifuged until clear. The clear supernatant liquid was transferred to sterile tubes. Agglutination test was performed with each serum and both Flexner and Shiga strains. (See Table 3.)

TABLE 3.—*Showing results of adsorption test of normal rabbit serum with Bacillus dysenteriæ, Shiga and Flexner types.*

I. SERUM ADSORBED BY SHIGA STRAIN 70.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Shiga 70	+++	+++	++	+	—	—	—	—
Flexner	++++	++++	+++	+	—	—	—	—

II. SERUM ADSORBED BY FLEXNER STRAIN 1.

Shiga 70	++	++	+	—	—	—	—	—
Flexner	+	—	—	—	—	—	—	—

III. UNTREATED SERUM.

Shiga 70	+++	+++	+	—	—	—	—	—
Flexner	++++	++++	+++	++	—	—	—	—

Other Shiga strains were tested with the adsorbed serum that remained from the previous test. Fresh twenty-four-hour-old acid agar cultures were used. One strain of Flexner was included also. The agglutination tests were made in exactly the same way as described in the previous test. The results were read next day. (See Table 4.)

TABLE 4.—*Showing results of adsorption test of normal rabbit serum with Bacillus dysenteriæ, Shiga and Flexner types.*

SERUM ADSORBED BY SHIGA STRAIN 70.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128
Shiga 68	++	++	—	—	—	—	—	—
Shiga 69	++	++	++	—	—	—	—	—
Shiga 71	++	++	++	++	++	++	++	++
Flexner 1	++++	+++	+	—	—	—	—	—

SERUM ADSORBED BY FLEXNER STRAIN 1.

Shiga 68	+	—	—	—	—	—	—	—
Shiga 69	+	—	—	—	—	—	—	—
Shiga 71	+	+	+	+	+	+	+	+
Flexner 1	—	—	—	—	—	—	—	—

UNTREATED SERUM.

Shiga 68	+++	+++	++	+	—	—	—	—
Shiga 69	++	+	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	+++
Flexner 1	++++	++++	++	—	—	—	—	—

Additional adsorption tests were performed, using other strains.

The pooled sera of four rabbits were divided into seven equal parts, and each part was placed in a sterile centrifuge tube. The tubes were marked according to the cultures that were to be used in the tests and were numbered from I to VI. The following cultures, planted the day before the test, were used in this experiment:

Shiga D. Flores.—This is a Shiga strain which was found agglutinable in normal rabbit serum, but nonagglutinable on heating for one or two hours.

Flexner 26.—This is a regular Flexner strain, highly agglutinable in normal rabbit serum, but nonagglutinable on heating.

Shiga 42.—This is a Shiga strain agglutinable in normal rabbit serum, but nonagglutinable on heating for one or two hours.

Shiga 2.—This strain behaves regularly and is nonagglutinable in normal rabbit serum and on heating.

Shiga 71.—This Shiga strain agglutinates in fresh normal rabbit serum. It also agglutinates spontaneously, as well as on heating.

The serum in each tube was diluted with an equal amount of sterile salt solution (0.9 per cent). Then the serum in tube I was absorbed with the Shiga culture D. Flores; tube II, with Flexner 26; tube III, with Shiga 42; tube IV, with Shiga 2; tube V, with Shiga 71. Tube VI received nothing but the serum and the salt solution and was marked "control serum." All the tubes were then placed in the incubator for one hour, after which time they were taken out and placed on top of the incubator and left there until next day.

On the next day, the serum in which Shiga 71 was emulsified was found completely agglutinated. The other sera were turbid, showing agglutination as indicated above. The untreated serum was clear.

All the tubes were centrifuged. The clear sera were transferred to other sterile centrifuged tubes and marked accordingly. They were treated in exactly the same way as was done the day before; that is, more bacteria were added to each tube. The next day there was complete agglutination in the tube marked Shiga 71; no agglutination in the other tubes was noticed.

The tubes were again centrifuged and the clear sera transferred to sterile tubes and kept in the ice box.

The procedure followed in these agglutination tests was the same as that described for the previous tests. Each of the adsorbed sera, including the control serum, was tested with each of the strains in question. Results were read next day. (See Table 5.)

Further adsorption tests were performed, using other strains. (See Tables 6 and 7.)

The adsorption experiments showed that one strain of Flexner adsorbed its own agglutinins, but none of the Shiga strains adsorbed the agglutinins of the Flexner strains tested. One Flexner strain adsorbed its own agglutinins, and all of those of the Shiga strains at times, while another strain of Flexner adsorbed its own agglutinins, but not those of the other Flexner strain nor those of the Shiga strains. From all these findings it is evident that the agglutination of Flexner strains behaves fairly regularly and constantly with normal rabbit serum, whereas the agglutination of the Shiga strains is not constant, the adsorption of Shiga agglutinins from normal rabbit serum being irregular even with the same strains. These findings would seem to indicate that such irregularities may be due to the quality of the Shiga antigen, particularly when we consider that the great majority of the smaller group of Shiga strains

TABLE 5.—*Showing results of adsorption test of normal rabbit serum with Bacillus dysenteriæ, Shiga and Flexner types.*

I. SERUM ADSORBED BY SHIGA STRAIN D. FLORES, SLIGHTLY IRREGULAR.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Control	Remarks.
Shiga D. Flores	+	+	±	—	—	—	—	—	—	—	—	—	
Flexner 26	++++	++++	++++	+++	+	±	—	—	—	—	—	—	
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 2	+	+	±	—	—	—	—	—	—	—	—	—	
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	+++	++++	++++	++++	Very small flakes throughout.

II. SERUM ADSORBED BY FLEXNER 26, REGULAR

Shiga D. Flores	—	—	—	—	—	—	—	—	—	—	—	—	
Flexner 26	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Sedimentation and very small flakes throughout.

III. SERUM ADSORBED BY SHIGA STRAIN 42, IRREGULAR.

Shiga D. Flores	—	—	—	—	—	—	—	—	—	—	—	—	
Flexner 26	+++	+++	+++	+++	+++	+	+	—	—	—	—	—	
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Small flakes.

215335—2

IV. SERUM ADSORBED BY SHIGA 2, REGULAR.

Shiga D. Flores	—	—	—	—	—	—	—	—	—	—	—	—	
Flexner 26	++++	++++	+++	+	—	—	—	—	—	—	—	—	
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Small flakes.

V. SERUM ADSORBED BY SHIGA 71, SPONTANEOUSLY AGGLUTINABLE.

Shiga D. Flores	—	—	—	—	—	—	—	—	—	—	—	—	
Flexner 26	++++	++++	+++	++	+	—	—	—	—	—	—	—	
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Small flakes.

VI. CONTROL SERUM, UNTREATED.

Shiga D. Flores	+++	++	+	+	+	±	—	—	—	—	—	—	
Flexner 26	++++	++++	++++	++++	+++	++	+	—	—	—	—	—	
Shiga 42	++	+	—	—	—	—	—	—	—	—	—	—	
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Small flakes.

TABLE 6.—*Showing results of adsorption of normal rabbit serum with Bacillus dysenteriæ, Shiga and Flexner types.*

SERUM ADSORBED BY FLEXNER 26, REGULAR FLEXNER.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Control.
Shiga Flores	++	++	—	—	—	—	—	—	—	—	—	—
Flexner 26	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 42	+	±	±	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

SERUM ADSORBED BY SHIGA 71, SPONTANEOUSLY AGGLUTINABLE.

Shiga Flores	++++	+++	+	—	—	—	—	—	—	—	—	—
Flexner 26	++++	++++	++++	+++	+	++	+	—	—	—	—	—
Shiga 42	+	+	—	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

CONTROL SERUM.

Shiga Flores	++++	+++	++	+	—	—	—	—	—	—	—	—
Flexner 26	++	+	±	—	—	—	—	—	—	—	—	—
Shiga 42	++	—	—	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

SERUM ADSORBED BY SHIGA FLORES.

Shiga Flores	+	++	+	—	—	—	—	—	—	—	—	—	—
Flexner 26	++++	++++	+++	++	—	—	—	—	—	—	—	—	—
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

SERUM ADSORBED BY SHIGA 42.

Shiga Flores	++	—	—	—	—	—	—	—	—	—	—	—	—
Flexner 26	++++	++++	++++	++++	+++	—	—	—	—	—	—	—	—
Shiga 42	+	+	+	—	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

TABLE 7.—*Showing results of adsorption test of normal rabbit serum with Bacillus dysenteriæ, Shiga and Flexner types.*

SERUM ADSORBED BY SHIGA 2.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	Control.
Shiga Flores	—	—	—	—	—	—	—	—	—	—	—	—
Flexner 26	++++	+++	++	+	±	—	—	—	—	—	—	—
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

CONTROL SERUM.

Shiga Flores	+	+	±	—	—	—	—	—	—	—	—	—
Flexner 26	++++	++++	+++	++	±	—	—	—	—	—	—	—
Shiga 42	±	+	+	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++

I. SERUM ADSORBED BY SHIGA 4, REGULAR SHIGA.

Shiga Flores	—	—	—	—	—	—	—	—	—	—	—	—
Flexner 26	++++	++++	+++	+	±	—	—	—	—	—	—	—
Shiga 42	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 2	—	—	—	—	—	—	—	—	—	—	—	—
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Flexner 1	+++	++	—	—	—	—	—	—	—	—	—	—
Flexner 12	+++	++	—	—	—	—	—	—	—	—	—	—
Shiga 4	+	—	—	—	—	—	—	—	—	—	—	—

II. SERUM ADSORBED BY FLEXNER 12, REGULAR FLEXNER.

Shiga Flores	++	+	±	−	−	−	−	−	−	−	−	−
Flexner 26	+++	+++	++	+	+	−	−	−	−	−	−	−
Shiga 42	++	++	+	−	−	−	−	−	−	−	−	−
Shiga 2	−	−	−	−	−	−	−	−	−	−	−	−
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Flexner 1	++	++	−	−	−	−	−	−	−	−	−	−
Flexner 12	−	+++	−	−	−	−	−	−	−	−	−	−

III. CONTROL SERUM.

Shiga Flores	+++	++	++	+	−	−	−	−	−	−	−	−
Flexner 26	+++	+++	+++	+++	−	−	−	−	−	−	−	−
Shiga 42	++	+	±	−	−	−	−	−	−	−	−	−
Shiga 2	+	−	−	−	−	−	−	−	−	−	−	−
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++
Flexner 1	++++	+++	+++	+	−	−	−	−	−	−	−	−
Flexner 12	++++	++++	+	−	−	−	−	−	−	−	−	−
Shiga 4	+	−	−	−	−	−	−	−	−	−	−	−

was found to be heat agglutinable, that the titer of the rabbit serum toward the Shiga strain is low, and especially when we consider the character of the agglutinate. As already mentioned, the sediment in the case of the Flexner strains is firmly packed, and when stirred shows mostly large flakes, whereas the sediment of the Shiga strains is loose, and the agglutinate consists of small microscopic flakes very similar to those found in the spontaneously agglutinable strains or in the strains agglutinated by boiling. This difference in the character of the agglutinate, observed early in these experiments, led us to undertake the qualitative antigen analysis, which disclosed the fact that the majority of the Shiga strains agglutinable by normal rabbit serum were heat agglutinable—a phenomenon which we are inclined to consider as a tendency on the part of the particular strain to spontaneous agglutinability brought about by boiling.

STUDY OF AGGLUTINATION AND ADSORPTION OF AGGLUTININS, USING POLYVALENT ANTIDYSENTERIC HORSE SERUM AND ALL AVAILABLE STRAINS OF BACILLUS DYSENTERIÆ

In order to substantiate this explanation the only thing for us to do was to extend our adsorption tests to immune serum. There is a statement [2] in the literature concerning agglutination to the effect that spontaneously agglutinable strains do not adsorb agglutinins of their kind. By means of this phenomenon it was hoped to demonstrate such relation as might exist between the spontaneously agglutinable and the heat agglutinable strains of the Shiga type of *B. dysenteriæ*. The phenomenon itself having been found with *B. typhosus*, it became necessary to learn whether it occurred also in the case of *B. dysenteriæ*. The immune serum used in these experiments was antidysenteric immune horse serum. It was prepared by subcutaneous injections of emulsions of eighteen Shiga cultures and twenty Flexner cultures. The Shiga cultures were the strains marked S 1 to S 18 in our tables.

The agglutination test (Table 8) with this serum shows that in a dilution 1 : 6 all of the strains included in this experiment were completely agglutinated, that is to say, twenty-one strains of regular Shiga; by regular Shiga we mean strains agglutinable by polyvalent antidysenteric serum, nonagglutinable by normal rabbit serum, and nonagglutinable on boiling

[2] Teague, Oscar, and Helen I. McWilliams, Journ. of Immunology 2 (1916–1917) 383–393.

for one hour. Other Shiga strains, designated as irregular, were also agglutinated completely; by irregular Shiga strains we mean strains agglutinable by polyvalent immune serum, more or less agglutinable by normal rabbit serum, and agglutinable or nonagglutinable on boiling one hour. It appears, therefore, that the polyvalent antidysenteric serum agglutinated not only the regular strains that were used in its preparation, but also the irregular ones that had not been injected into the horses.

TABLE 8.—*Showing the results of adsorption tests of polyvalent antidysenteric serum by regular, irregular, and spontaneously agglutinable strains of Bacillus dysenteriæ Shiga.*

[Dilution of serum, 1:6.]

Strain No.	Serum adsorbed by S 2.	Serum adsorbed by S 70.	Serum adsorbed by S 71.	Untreated serum.	Control.	Remarks.
S 1	—	++++	++++	++++	—	R
S 2	—	++++	++++	++++	—	R
S 3	—	++++	++++	++++	—	R
S 4	—	++++	++++	++++	—	R
S 5	—	++++	++++	++++	—	R
S 6	—	++++	++++	++++	—	R
S 7	—	++++	++++	++++	—	R
S 8	—	++++	++++	++++	—	R
S 9	—	++++	++++	++++	—	R
S 10	—	++++	++++	++++	—	R
S 11	—	++++	++++	++++	—	R
S 12	—	++++	++++	++++	—	R
S 13	—	++++	++++	++++	—	R
S 14	—	++++	++++	++++	—	R
S 15	—	++++	++++	++++	—	R
S 16	—	++++	++++	++++	—	R
S 17	—	++++	++++	++++	—	R
S 18	—	++++	++++	++++	—	R
918–2	—	++++	++++	++++	—	R
125140	—	++++	++++	++++	—	R
Stock Shiga	—	++++	++++	++++	—	R
S 42	—	++++	++++	++++	—	RS+ B—
S 43	++++	++++	++++	++++	—	RS+ B+
S 44	—	++++	++++	++++	—	RS+ B+
S 56	++++	++++	++++	++++	—	RS+ B+
S 59	++++	++++	++++	++++	—	RS+ B+
S 60	++++	++++	++++	++++	—	RS+ B+
S 66	++++	++++	++++	++++	—	RS+ B+
S 67	++++	++++	++++	++++	+	RS+ B+
S 69	—	++++	++++	++++	—	RS+ B+
S 70	++++	++++	++++	++++	—	RS+ B+
S 71	++++	++++	++++	++++	++++	Spontaneously agglutinable.
D. Flores	—	++++	++++	++++	—	RS+ B—
Suzuki	++++	++++	++++	++++	—	RS+ B—
Salvatierra	+++	++++	++++	++++	—	RS+ B—
Palisoc	+	++++	++++	++++	—	RS+ B—

The adsorption test showed that one regular Shiga strain adsorbed agglutinins for all regular Shiga strains and also for four of the irregular strains. Whether this adsorption was complete or not in the case of the irregular strains is not evident from this experiment, because only one dilution of the serum was used.

However, when the polyvalent antidysenteric serum was adsorbed by an irregular strain (that is, a strain which was agglutinable both by rabbit normal serum and on boiling) no adsorption of agglutinins took place, either for the regular strains or for the irregular ones. The strain agglutinable by normal rabbit serum and on boiling behaved in this respect in the same way as did the spontaneously agglutinable strain.

A quantitative agglutination test was made using the polyvalent antidysenteric horse serum and three serologically regular Shiga strains, four irregular Shiga strains (that is to say, agglutinable by normal rabbit serum and on boiling), one slightly irregular strain (that is, agglutinable by normal rabbit serum but not on boiling), and one spontaneously agglutinable Shiga strain. It is interesting to note that the regular Shiga strains that were used for the production of the serum were agglutinated to a lower degree than were the irregular ones that were not used for the immunization of the horses. The slightly irregular Shiga strain gave about the same titer as did the regular ones.

When the serum was adsorbed by any of the three regular Shiga strains, the agglutinins for the regular ones disappeared completely, while those for the irregular ones only partially disappeared. The irregular strains adsorbed partially for the irregular ones and not at all for the regular ones. The slightly irregular strain, on the other hand, adsorbed partially for the regular strains as well as for the irregular ones.

In the next experiment (Table 10) one portion of the polyvalent immune horse serum, referred to above, was adsorbed by the regular Shiga strain, and another portion by the regular Flexner strain. The agglutination test with the untreated polyvalent serum showed that the regular Shiga and the Flexner strains were agglutinated to about the same dilution. When the serum was adsorbed by the regular Shiga, it decreased the titer for Flexner very slightly, whereas it lost the agglutinins for regular Shiga completely and also partially for the irregular strain and for the slightly irregular strain.

TABLE 9.—*Showing the results of adsorption tests of polyvalent antidysenteric horse serum by various strains of Bacillus dysenteriæ Shiga.*

CONTROL SERUM, UNTREATED.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	Control.	Remarks.
S 59	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	—	—	—	—	RS+ B+?
S 60	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	—	—	—	—	RS+ B+
S 66	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	—	—	RS+ B+
S 1	++++	++++	++++	++++	++	+	—	—	—	—	—	—	—	—	RS— B—
S 2	++++	++++	++++	++++	++	+	—	—	—	—	—	—	—	—	RS— B—
S 3	++++	++++	++++	++++	+++	++	—	—	—	—	—	—	—	—	RS— B—
S Flores	++++	++++	++++	++++	+	—	—	—	—	—	—	—	—	—	RS+ B—
S 70	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	++	—	RS+ B+
S 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Spontaneously agglutinable.

SERUM ADSORBED BY S 1, REGULAR SHIGA; DOES NOT AGGLUTINATE WITH RABBIT SERUM OR ON BOILING.

S 60	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	—	RS+ B+
S 66	++++	++++	+++	++	+	—	—	—	—	—	—	—	—	—	RS+ B+
S 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—

SERUM ADSORBED BY S 2, REGULAR SHIGA; DOES NOT AGGLUTINATE WITH RABBIT SERUM OR ON BOILING.

S 60	++++	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	—	RS+ B+
S 66	++++	++++	++++	+++	—	—	—	—	—	—	—	—	—	—	RS+ B+
S 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 70	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++	—	—	—	RS+ B+
S 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Spontaneously agglutinable.

TABLE 9.—*Showing the results of adsorption tests of polyvalent antidysenteric horse serum by various strains of Bacillus dysenteriæ Shiga*—Continued.

SERUM ADSORBED BY S 3, REGULAR SHIGA; DOES NOT AGGLUTINATE WITH RABBIT SERUM OR ON BOILING.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	1:4096	Control.	Remarks.
S 60	++++	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	RS+ B+
S 66	++++	++++	++++	++++	+	—	—	—	—	—	—	—	—	—	RS+ B+
S 1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
S 3	—	—	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—

SERUM ADSORBED BY S 59, IRREGULAR SHIGA; AGGLUTINATES WITH RABBIT SERUM AND ON BOILING.

S 59	++++	++++	++	—	—	—	—	—	—	—	—	—	—	—	RS+ B+?
S 60	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	—	—	RS+ B+
S 66	++++	++++	++++	++++	+++	++	+	—	—	—	—	—	—	—	RS+ B+
S 1	++++	++++	++++	++++	++++	+	—	—	—	—	—	—	—	—	RS— B—
S 2	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	—	RS— B—
S 3	++++	++++	++++	++++	++++	+++	—	—	—	—	—	—	—	—	RS— B—

SERUM ADSORBED BY S 60, IRREGULAR SHIGA; AGGLUTINATES WITH RABBIT SERUM AND ON BOILING.

S 59	++++	++++	++++	++++	++++	++++	++++	++++	+++	++	—	—	—	—	RS+ B+?
S 60	++++	++++	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	RS+ B+
S 66	++++	++++	++++	++++	++++	+++	++	—	—	—	—	—	—	—	RS+ B+
S 1	++++	++++	++++	++++	++++	+++	++	—	—	—	—	—	—	—	RS— B—
S 2	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	—	—	RS— B—
S 3	++++	++++	++++	++++	++++	+++	—	—	—	—	—	—	—	—	RS— B—

SERUM ADSORBED BY S 66, IRREGULAR SHIGA; AGGLUTINATES WITH RABBIT SERUM AND ON BOILING.

S 59	++++	++++	++++	++++	++++	++++	++	+	—	—	—	—	—	—	RS+	B+?
S 60	++++	++++	++++	++++	++++	++++	+++	++	+	—	—	—	—	—	RS+	B+
S 66	++++	++++	++++	++++	++++	++++	+++	—	—	—	—	—	—	—	RS+	B+
S 1	++++	++++	++++	++++	++++	+++	—	—	—	—	—	—	—	—	RS—	B—
S 2	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	—	RS—	B—
S 3	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	—	RS—	B—

SERUM ADSORBED BY SHIGA 70, IRREGULAR SHIGA; AGGLUTINATES WITH RABBIT SERUM AND ON BOILING.

Shiga 2	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	—	—	RS—	B—
Shiga 70	++++	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	+	—	—	RS+	B+
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Spontaneously agglutinable.	

SERUM ADSORBED BY SHIGA 71, IRREGULAR SHIGA, SPONTANEOUSLY AGGLUTINABLE.

Shiga 2	++++	++++	++++	+++	++	+	±	—	—	—	—	—	—	—	RS—	B—
Shiga 70	++++	++++	++++	++++	++++	++++	++++	++++	+++	+++	+++	++	—	—	RS+	B+
Shiga 71	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	++++	Spontaneously agglutinable.	

SERUM ADSORBED BY S. FLORES, SLIGHTLY IRREGULAR SHIGA; AGGLUTINATES WITH RABBIT SERUM, BUT NOT ON BOILING.

S 60	++++	++++	++++	++++	++++	+++	+	—	—	—	—	—	—	—	RS+	B+
S 66	++++	++++	+++	+	—	—	—	—	—	—	—	—	—	—	RS+	B+
S 1	++++	+++	—	—	—	—	—	—	—	—	—	—	—	—	RS—	B—
S 2	++++	+++	+	—	—	—	—	—	—	—	—	—	—	—	RS—	B—
Flores	+++	++	++	—	—	—	—	—	—	—	—	—	—	—	RS+	B—

TABLE 10.—*Showing the results of adsorption tests of polyvalent antidysenteric serum by regular strains of Bacillus dysenteriæ Shiga and B. dysenteriæ Flexner.*

CONTROL SERUM, UNTREATED.

	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:256	1:512	1:1024	1:2048	Control.	Remarks.
S 1	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	—	RS— B—
Flexner 12	++++	++++	++++	++++	++++	+++	++	—	—	—	—	—	—	RS+ B—
S Flores	++++	++++	++++	++++	++++	++++	++	+	—	—	—	—	—	RS+ B—
S 70	++++	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	RS+ B+

SERUM ADSORBED BY FLEXNER 12, REGULAR FLEXNER; AGGLUTINATES WITH RABBIT SERUM, BUT NOT ON BOILING.

S 1	++++	++++	++++	++++	++++	++++	++	—	—	—	—	—	—	RS— B—
Flexner 12	+	+	+	+	+	—	—	—	—	—	—	—	—	Small flakes.
														RS+ B—
S Flores	++++	++++	++++	++++	++++	+	—	—	—	—	—	—	—	RS+ B—
S 70	++++	++++	++++	++++	++++	+++	—	—	—	—	—	—	—	RS+ B+

SERUM ADSORBED BY S 1, REGULAR SHIGA; DOES NOT AGGLUTINATE WITH RABBIT SERUM OR ON BOILING.

S 1	+	—	—	—	—	—	—	—	—	—	—	—	—	RS— B—
Flexner 12	++++	++++	++++	++++	++++	+	—	—	—	—	—	—	—	RS+ B—
S Flores	++++	+++	++	+	±	—	—	—	—	—	—	—	—	RS+ B—
S 70	+++	+++	+++	+	—	—	—	—	—	—	—	—	—	RS+ B+

When adsorbed with the regular Flexner strain, the polyvalent serum agglutinated the regular Shiga as well as the irregular and the slightly irregular to about the same degree as the untreated serum. The Flexner strain adsorbed completely the specific agglutinins for Flexner but left the small flake agglutinins preserved.

This finding is of interest. The fact that the majority of the irregular Shiga strains were agglutinated to a higher degree than were the regular ones and, further, that they did not adsorb the agglutinins of the regular strains or their own completely, pointed to the possibility that the irregular Shiga strains had highly developed Flexner receptors and that the residual agglutinins were the Flexner agglutinins because the serum was polyvalent. The adsorption test with the Flexner strain of the polyvalent serum shows, however, that this is not the case and that the inability of the irregular strains to adsorb agglutinins of their group and their own is due to the change in antigen or its receptor system. The slightly irregular strain behaves again more like the regular ones than the strains which proved agglutinable on boiling and with normal rabbit serum.

STUDY OF THE QUALITY OF ANTIGEN OF BACILLUS DYSENTERIÆ BY ACID AGGLUTINATION (MICHAELIS)[3]

Further to substantiate our view we undertook a series of tests tending to show the influence of ion concentration on the agglutinability of all of the various strains of *Bacillus dysenteriæ* in our collection.

The method employed was the acid agglutination, as introduced by Michaelis. Six solutions were made according to the scheme given below. A distilled water control and physiological salt solution controls were included in the test. It was found that the maximum of agglutination with the regular strains took place in solutions V and VI. The line of demarcation with the regular Shiga strains is rather sharp, and agglutination occurs in the tubes of highest ion concentration. The regular Flexner strain behaved in the same way, with a few exceptions, where small flakes were found in solutions containing lower concentration than that in solution V. With the irregular strains the range of agglutination was shifted to the left, and distinct agglutination was found in tubes containing a

[3] Kolle, W., and H. Metsch, Die experimentelle Bakteriologie, etc., 6th ed. 1 (1922) 184.

lower ion concentration than that in tube V. The spontaneously agglutinable strain and six other irregular Shiga strains gave complete agglutination in all of the tubes and, strange to say, Flexner's strains isolated from carriers behaved similarly with regard to acid agglutination.

These findings would seem to be confirmatory evidence of our view expressed above.

TECHNIC

The acid agglutination was carried out in the following manner: The bacterial suspensions were made in distilled water and each tube received 0.5 cubic centimeter of the suspension and 0.5 cubic centimeter of the corresponding solution. The reading was taken on the next day.

The scheme of solutions used for acid agglutination tests was as follows:

	cc.
Solution I:	
Normal sodium hydroxide	5
Normal hydrochloric acid	7.5
Water	87.5
Solution II:	
Normal sodium hydroxide	5
Normal hydrochloric acid	10
Water	85
Solution III:	
Normal sodium hydroxide	5
Normal hydrochloric acid	15
Water	80
Solution IV:	
Normal sodium hydroxide	5
Normal hydrochloric acid	25
Water	70
Solution V:	
Normal sodium hydroxide	5
Normal hydrochloric acid	45
Water	50
Solution VI:	
Normal sodium hydroxide	5
Normal hydrochloric acid	85
Water	10
Solution VII:	
Control	
Distilled water	
Solution VIII:	
Normal salt solution, 0.9 per cent	
Control	

A series of eighteen regular Shiga strains was subjected to the acid agglutination test. It can be seen from Table 11, in

which the results are tabulated, that the regular strains behaved fairly uniformly. Although the agglutination was not complete, the maximum with all strains was noticed in solutions V and VI. Furthermore, the range of agglutination is sharply defined.

TABLE 11.—*Showing the results of acid agglutination (regular Shiga strains).*

	Solution I.	Solution II.	Solution III.	Solution IV.	Solution V.	Solution VI.	Control.	Remarks.
S 1	—	—	+	++	++	+	—	RS— B—
S 2	—	—	—	+	++	+	—	RS— B—
S 3	—	—	—	++	++	++	—	RS— B—
S 4	—	—	—	+	++	+	—	RS— B—
S 5	—	—	—	+	++	++	—	RS— B—
S 6	—	—	—	+	++	++	—	RS— B—
S 7	—	—	—	++	++	++	—	RS— B—
S 8	—	—	—	+	++	++	—	RS— B—
S 9	—	—	—	+	+	++	—	RS— B—
S 10	—	—	—	+	++	++	—	RS— B—
S 12	—	—	—	—	+	++	—	RS— B—
S 13	—	—	—	—	+	++	—	RS— B—
S 14	—	—	—	—	±	++	—	RS— B—
S 15	±	—	—	—	+	++	—	RS— B—
S 16	—	—	—	—	+	+	—	RS— B—
S 17	—	—	—	—	++	++	—	RS— B—
S 18	—	—	—	—	++	++	—	RS— B —

The irregular strains (Table 12), however, behaved differently, in as much as the majority of them agglutinated far more strongly than did the regular ones, and they usually had a much wider range of positive agglutination; or, in other words, the optimum of positive agglutination shifted toward the end of the lower ion concentration. Several behaved similarly to the spontaneously agglutinable strain, in as much as complete or almost complete agglutination took place in all of the six solutions. They are not, however, as sensitive as is the spontaneously agglutinable strain which, though it remains in even suspension in distilled water, is brought to flocculation by the mere presence of 0.9 per cent of sodium chloride.

For the sake of completeness, rather than to bring more evidence of the correctness of our view, expressed above, the acid agglutination test was also applied to all the available strains of mannite fermenters (Table 13). They were all regular Flexner strains (that is, agglutinable by normal rabbit serum and nonagglutinable on boiling for one hour) with one exception (I), which was agglutinable on boiling; and, although this

TABLE 12.—*Showing the results of acid agglutination (thirteen irregular Shiga strains).*

	Solution I.	Solution II.	Solution III.	Solution IV.	Solution V.	Solution VI.	H_2O control.	Control emulsion.	Remarks.
S 42	+++	+++	+++	+++	+++	+++	—	—	RS+ B—
S 43	—	+	+	++++	+	—	—	—	RS+ B+
S 44	—	±	±	+++	++	±	—	—	RS+ B+
S 56	—	—	+	++++	++	++	—	—	RS+ B+
S 59	—	—	±	+	±	±	—	—	RS+ B+?
S 60	—	—	+	++	++	±	—	—	RS+ B+
S 66	±	±	±	++	+	±	—	—	RS+ B+
S 67	+++	++++	++++	++++	++++	++++	—	—	RS+ B+
S 68	—	±	±	±	±	—	—	—	RS+ B+
S 69	—	±	+	+	+	±	—	—	RS+ B+
S 70	++++	++++	++++	++++	++++	++++	—	—	RS+ B+
S Flores	—	—	++	++	++	++	—	—	RS+ B—
S 71	++++	++++	++++	++++	++++	++++	—	++++	RS+ B+

strain showed only a slight degree of agglutination, the range of agglutinability extended over all of the six solutions. The general tendency with mannite fermenters is to have the optimum ion concentration corresponding to solutions VI, V, and IV. Some of them extended farther to the lower ion concentration but showed no irregularity in normal rabbit serum or on boiling.

TABLE 13.—*Showing the results of acid agglutination (regular Flexner strains).*

	Solution I.	Solution II.	Solution III.	olution IV.	Solution V.	Solution VI.	Control VII.	Remarks.
Fl. 1	+	+	+	+	+	+	—	RS+ B+
Fl. 2	—	—	+	+++	+++	++	—	RS+ B—
Fl. 3	+	+	+	+	+	+	—	RS+ B—
Fl. 4	—	—	+	+++	+++	++	—	RS+ B—
Fl. 9	—	—	+	++	++	++	—	RS+ B—
Fl. 10	—	—	+	+	+++	+	—	RS+ B—
Fl. 11	—	—	+	++	++	++	—	RS+ B—
Fl. 12	—	—	+	+++	+++	++	—	RS+ B—
Fl. 14	—	—	—	++	+++	++	—	RS+ B—
Fl. 15	—	—	—	++	+++	++	—	RS+ B—
Fl. 16	—	+	+++	+++	+++	+++	—	RS+ B—
Fl. 17	—	—	+	++	+++	++	—	RS+ B—
Fl. 18	—	—	++	+++	+++	++	—	RS+ B—
Fl. 19	+	++	+++	+++	+++	+++	—	RS+ B—
Fl. 20	—	—	+	+++	+++	+++	—	RS+ B—
Fl. 21	—	—	—	+	++	++	—	RS+ B—
Fl. 22	—	—	—	++	++	++	—	RS+ B—
Fl. 23	—	+	+	++	+++	++	—	RS+ B—
Fl. 24	—	—	—	+	++	++	—	RS+ B—
Fl. 25	—	—	—	++	++	++	—	RS+ B—
Fl. 26	—	—	—	—	±	—	—	RS+ B—
Fl. 39	—	—	+	+++	+++	+++	—	RS+ B—
Fl. 40	—	—	—	+++	+++	++	—	RS+ B—
Fl. 41	—	—	—	+	++	++	—	RS+ B—

SUMMARY

Two types of *Bacillus dysenteriæ*, Shiga and Flexner were subjected to agglutination tests by normal sera of the following animals: Guinea pig, rabbit, horse, mule, goat, sheep, and monkey. It was found that the sera of horse, mule, sheep, and goat agglutinate both the Flexner and the Shiga types of *B. dysenteriæ*. The rabbit serum behaved peculiarly, in as much as it agglutinated promptly all the Flexner strains available, forming large flakes and firmly packed sediment.

The majority of the Shiga strains available (that is, strains isolated locally from patients and carriers as well as cultures

obtained from abroad) were nonagglutinable by normal rabbit serum, while a smaller number of Shiga strains were agglutinated by normal rabbit serum. The largest number of the latter were agglutinable by boiling.

In the adsorption experiments the Flexner type adsorbed the agglutinins for the corresponding strains regularly, from normal rabbit serum, whereas the Shiga strains showed irregular results; that is, serum adsorbed by the Shiga strains did not adsorb the Flexner agglutinins, whereas sometimes it agglutinated the Shiga strains and sometimes it did not.

In view of the character of the agglutinate (that is, mostly small flakes and loose sediment) and in view of the irregularity of adsorption and, particularly, of the fact that the majority of these Shiga strains are agglutinable on boiling, the agglutination of Shiga strains in general by normal rabbit serum is believed to be due to the quality of the antigen of certain strains rather than to the presence of real agglutinins such as one finds for Flexner strains in normal rabbit serum.

Our smaller group of mannite nonfermenters agrees remarkably with the small group in our collection which was found to represent a serologic small group when monovalent serum was used and to produce indol, thus resembling the Flexner strains in this respect (Lacy).[4] The small group was found to be agglutinable by the polyvalent immune horse serum to a higher degree than were the regular strains, although the regular strains were used exclusively for the preparation of the serum.

The majority of the small group (that is, the strains agglutinable by normal rabbit serum) were found to be spontaneously agglutinable on boiling for one hour in a suspension of salt solution. There were, however, some members of this group that were agglutinable by normal rabbit serum, but were not agglutinable on boiling.

When the adsorption test of the polyvalent immune horse serum was performed the regular strains showed reciprocity in complete adsorption of agglutinins within their group and adsorbed partially the agglutinins for the irregular strains. The members of our small group adsorbed partially their own agglutinins but did not adsorb the agglutinins of the regular

[4] Philip. Journ. Sci. 28 (1925) 313–328.

strains. Two strains were encountered that were agglutinable by normal rabbit serum, but not on boiling, and which adsorbed partially agglutinins for both the regular and the irregular Shiga strains, thus forming an intermediate stage between the regular and the irregular strains. It is interesting to note that these strains, according to the classification by monovalent serum, belong to the larger group (Lacy).[5] Another strain, spontaneously agglutinable in salt solution suspension, adsorbed neither for the regular nor for the irregular strain, thus forming the extreme end of the serological stages of mannite nonfermenters. In agreement with this serologic grouping by the use of qualitative antigen analysis and normal agglutinins, the regular Shiga strains showed a narrow, sharply defined zone of agglutination when subjected to the acid agglutination test; that is, the agglutination was restricted and most pronounced in the solutions of highest ion concentration. The irregular Shiga strains, that is, agglutinable by normal rabbit serum and agglutinable or nonagglutinable by boiling, showed as a rule a more pronounced agglutination and a wide range of positive agglutination not infrequently extending over all of the six solutions used.

The mannite fermenters on the average showed a similar optimum of acid agglutination as did the nonfermenters, but the range was somewhat wider than that in Shiga strains.

DISCUSSION

If we consider the findings presented in this paper and use them as a basis for interpretation of the serological grouping of mannite nonfermenters, as offered by Lacy,[6] we can hardly escape the impression that the gradual degeneration of the antigen of the bacteria of one group is responsible for the differences in agglutinability of the various strains and the consequent serologic grouping.

As rightly pointed out by Lacy the preliminary test of identification of *Bacillus dysenteriæ* by agglutination with monovalent agglutinant serum is not advisable, in as much (as Lacy has shown) as the small group would have escaped the attention of the bacteriologist diagnostician. From our results, however, it can be seen that the polyvalent antidysenteric horse serum,

[5] Loc. cit. [6] Loc. cit.

as prepared by us for therapeutic purposes and used as a preliminary identification test of suspicious colonies on plates, agglutinates the members of the small group to at least as high a degree as its homologous strains and frequently to a much higher degree.

In our experiments the regular Shiga strains were found resistant to agglutination by serum of their own to a higher degree than were the irregular ones. The sensitiveness to agglutination of the latter by the serum of the former is expressed by their being more highly agglutinable by the serum of the regular strains than are the regular strains themselves. This is further emphasized by the fact that they are agglutinable by normal rabbit serum and more so by the fact that the majority of the strains are agglutinable on boiling for one hour.

Thus we have a series of stages of susceptibility to agglutination from the regular Shiga strains that are comparatively slightly agglutinable by homologous immune horse serum, nonagglutinable by normal rabbit serum, and nonagglutinable on boiling; have a sharply defined optimum of acid agglutination restricted to the solutions of highest ion concentration; adsorb completely agglutinins of their own as well as of all members of their group and adsorb partially those of the irregular strains of mannite nonfermenters, to the extreme other end, represented by the spontaneously agglutinable strain so susceptible to agglutination that the mere presence of the small amount of sodium chloride in physiologic salt solution will bring about agglutination. All degrees of gradual degeneration are represented in our collection, between the regular strains and the spontaneously agglutinable strains. There are strains which agglutinate by rabbit serum only and not on boiling and have a wide range of agglutination in the acid agglutination test, and those which agglutinate on boiling as well as by rabbit serum and show high susceptibility to acid agglutination. Some of these adsorb their own agglutinins and those of their group, while others behave with respect to adsorption as well as to acid agglutination in exactly the same manner as does the spontaneously agglutinable strain.

Hand in hand with the increase of susceptibility to agglutination there goes a decrease in their adsorbing capacity. We find that an immune horse serum prepared with the regular strains agglutinates all the Shiga strains in our collection; but

the adsorption experiment shows the fine serologic distinction between the two groups. In connection with these observations we must not omit to mention the findings of Morishima [7] who, working in the Bureau of Science on the constancy of types of *Bacillus dysenteriæ*, found that the serum prepared by the original mother culture agglutinated itself as well as the variant. The serum prepared by the variant agglutinated the variant itself and the mother culture; but, while the mother culture adsorbed agglutinins of its own as well as those for the variant, the variant adsorbed the agglutinins for itself only, but not those for the mother culture.

It is evident that there is a partial loss in the receptor system of certain serologic groups of *Bacillus dysenteriæ*, while the changes as noticed by Morishima in mannite fermenters were not only serologic, but also cultural, in as much as it was possible to change the "Y" type into a Flexner type and vice versa; it was impossible to induce mannite nonfermenters to change their fermenting properties. In our case the only explanation is that the irregular strains of mannite nonfermenters have gradually variated, but the mannite nonfermenters, being very stable as far as fermentation of carbohydrate is concerned, gradually and partially lost their adsorbing receptor system but retained their fermentative properties intact. The grouping of our collection into serologic groups by Lacy [8] finds its explanation in our experiments and in those of Morishima.[9]

In accordance with the receptor theory of Ehrlich, we have to assume three systems of receptor; one, which causes the emulsion to agglutinate when brought in contact with immune serum; another, which adsorbs the agglutinins from the immune serum; and a third, which stimulates the production of agglutinins when injected into the animal's body (agglutinogen).

The agglutinogenic property of our strains has not been tested by us, in view of the fact that this part of the work has been done by Lacy with a fairly representative number of the members of our collection. We can, therefore, use the results of his investigation to advantage, as far as the explanation of our results is concerned. From the accompanying table (Table 14) the remarkable agreement between the grouping by Lacy

[7] Philip. Journ. Sci. 29 (1926) 447–463.
[8] Philip. Journ. Sci. 25 (1925) 313–328.
[9] Philip. Journ. Sci. 29 (1926) 447–463.

TABLE 14.—*Showing the results of our classification of Bacillus dysenteriæ Shiga strains and that made by G. R. Lacy, using the same strains.*

Grouping based on quality of antigen and normal agglutinins (Schöbl and Villasamil).			Grouping based on agglutinogenic property and monovalent agglutinins (Lacy).		
Regular group.	Slightly irregular group.	Irregular group.	I, large group.	II, small group.	III, subgroup.
Strain.	*Strain.*	*Strain.*	*Strain.*	*Strain.*	*Strain.*
1	42	43	1	43	Espiritu.
2	Flores.	44	2	44	Salvatierra.
3		56	3	56	Reyes.
4		59	4	59	Mendiola.
5		60	5	60	F. Pili.
6		66	6	66	
7		67	7	Suzuki.	
8		68	8		
9		69	9		
10		70	10		
11		71	11		
12		Suzuki.	12		
13		Espiritu.	18		
14		Salvatierra.	42		
15		Reyes.	Flores.		
16					
17					
18					
Mendiola.					
F. Pili.					

of the mannite nonfermenting group of *Bacillus dysenteriæ* (based on the agglutinogenic property of the strains) and our grouping (based on the quality of antigen and normal agglutinins) is at once evident. Our slightly irregular strains (Flores 42) belong in the large group of Lacy, while the members of Lacy's subgroup, which agglutinated neither with the large group nor with the small group of sera, are distributed over our regular and irregular groups.

STUDIES ON THE SEROLOGY OF LEPROSY, I

THE WASSERMANN REACTION IN LEPROSY [1]

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INTRODUCTION

Leprosy has long been considered as preëminently the non-treponematous disease that yields a positive Wassermann reaction. The majority of workers who have investigated the matter have affirmed this. A peculiar feature is that there are wide differences in the reports as to the frequency of positive reactions, the percentages varying from as low as 20 to practically 100. Further, in many instances fixation is incomplete. Therefore, it is evident that the situation is very different from that in syphilis and yaws. Comparatively recently the contrary view has been advanced; namely, that leprosy, uncomplicated, does not give rise to positive reactions. However, most of those interested in the subject are under the earlier impression.

It was felt that the matter should be investigated further with the view to determining which view should be adopted. This is of importance, quite aside from any possible value of the reaction in the diagnosis of doubtful cases of leprosy and from the theoretic interest in view of the otherwise practical specificity of the reaction; for the reaction can be used as an indication and guide for the necessary treatment of complicating syphilis or yaws in lepers only in case there is assurance that it is not caused by leprosy itself. Furthermore, this information was desired by the staff of the pathological section of the Culion Leper Colony, in connection with projected studies on other phases of the serology of leprosy. The findings in five hundred

[1] Published on recommendation of the Philippine Leprosy Research Board and with the approval of the Director of Health.

cases are here given, together with a consideration of the effect of antitreponematous therapy in certain of the positive cases.

LITERATURE

It would be fruitless repetition to review extensively the literature on the subject of the Wassermann reaction; this can be found in a recent paper by Kolmer and Denney.[2] Of the papers reporting positive results we will cite only those that have been published recently, as they are fairly representative.

Goodpasture,[3] working in the Philippines, reports positive results in eight (62 per cent) of thirteen untreated cases and in eleven (85 per cent) of thirteen undergoing chaulmoogra treatment that were still clinically and bacteriologically positive. In sixteen cases that had become clinically and bacteriologically negative as a result of treatment, he found the reaction uniformly negative. He believes, therefore, that a positive Wassermann reaction in leprosy is due to infection with the bacillus of leprosy, and that the disappearance of the reaction is a specific phenomenon associated with clinical improvement and diminution in the number of the acid-fast bacilli.

Lloyd, Muir, and Mitra[4] report from India that they found the reaction completely positive in 41.7 per cent of two hundred twenty-eight cases examined; if the partially positive reactions are included, this figure is brought to 53.5 per cent. Of twenty-seven purely nodular cases 70.4 per cent were positive in some degree; of one hundred sixteen mixed cases, 62.9 per cent; and of eighty-five anæsthetic cases, 35.2 per cent. In fifty-eight children examined, 81 per cent were completely or partially positive, including all of four nodular cases, all but one of twenty mixed cases, and 70 per cent of thirty-four anæsthetic cases.

Later, however, in their study on the effect of antisyphilitic treatment on the reaction in leprosy[5] these authors conclude that many of the cases were suffering from syphilis also, but they apparently still believe some of the positive reactions to be due to leprosy. Recently, Muir stated[6] that, with further treatment of these cases, the apparent influence of syphilis in this connection is becoming greater.

[2] Arch Derm. & Syph. 8 (1923) 63.
[3] Philip. Journ. Sci. 22 (1923) 428.
[4] Ind. Journ. Med. Res. 11 (1923) 1.
[5] Ind. Journ. Med. Res. 12 (1924) 213–220.
[6] Personal communication.

Schöbl and Basaca,[7] in a study of the distilled water (globulin) precipitation reaction in leprosy, performed the Wassermann reaction in parallel with that test and obtained about 35 per cent positives. However, most of the reactions were very weak, for eight of the eleven were plus-minus, and two were 1-plus.

Of the many reports on this subject very few record failure to obtain positive results. So far as we are aware, Bloombergh [8] was the first to question the occurrence of the reaction in leprosy. He examined twenty-one cases and in only three was it positive. Of these, one gave a history of syphilis; the other two were unfortunately lost sight of. He, therefore, considered it doubtful that a positive reaction is to be obtained as a result of infection with the bacillus of leprosy.

Ten years ago Mathis and Baujean [9] definitely stated that the Wassermann reaction is negative in cases of pure leprosy. Their work was apparently ignored by subsequent workers, and Mathis [10] presented another paper at the recent leprosy conference in Strassbourg, confirming the previous report. The technic of Calmette and Massol was used in this work.

Kolmer and Denney [11] report uniformly negative results in nonsyphilitic cases with the former's new standardized technic, though with an old technic about 7 per cent of the same sera were positive.

TECHNIC

Two methods were employed by us. All of the sera were tested by the new standardized method of Kolmer [12] and most of them by this method alone. This was primarily chosen, both because of the results with it in leprosy reported by Kolmer and Denney, and because it is admitted by several workers [13] to be superior as regards the diagnosis of syphilis. It

[7] Philip. Journ. Sci. 25 (1924) 1.

[8] Philip. Journ. Sci. § B 6 (1911) 335.

[9] Bull. Soc. Path. Exot. 8 (1915) 252.

[10] La reaction de Wassermann dans la lépre. Troisieme Conference International Scientifique de la Lépre, Strassbourg, 28–31 Juillet, 1923, Paris, J. B. Balliere et Fils, 19 Rue Hautejouille (1924) 229–231.

[11] Arch. Derm. & Syph. 8 (1923) 63.

[12] Am. Journ. of Syphilis, VI, 1 (1922).

[13] Shivers, C. H. de T., Arch. Derm. & Syph. 6 (1922) 344; Kilduffe, R. A., Arch. Derm. & Syph. 6 (1922) 709; Palmer, L. J., and W. E. Gibbs, Arch. Derm. & Syph. 6 (1922) 738; Irvine, H. G., and D. Stern, Arch. Derm. & Syph. 8 (1923) 818; Schamberg, J. F., and S. S. Greenbaum, Journ. Am. Med. Assoc. 80 (1923) 836–838.

would seem that, if in uncomplicated cases of leprosy there is produced a substance related to the reagin produced in treponematous infections, this method would be the most likely to demonstrate its presence and to eliminate false positives due to unrelated serum bodies. For comparison with this method, one hundred fifty sera were tested in parallel with an ordinary technic, which need not be described in detail. In this, both a plain and a cholesterinized alcoholic extract of lean beef heart were used.

The Kolmer antigen was used as recommended; namely, in a dose containing ten antigenic units, each unit one-thirtieth of the anticomplementary unit. The plain and cholesterinized alcoholic antigens of the ordinary technic were closely titrated and two and a half antigenic units were used which amounted, with the plain alcoholic antigen, to one-fourth of the anticomplementary unit, and with the cholesterinized, one-fifth. A closely adjusted antisheep hæmolytic system was employed. The hæmolysin used at the beginning of the work was a potent one, of a titer of 1: 8,000. Later, the hæmolysin that we produced had a titer of only 1: 2,000. Two units in 0.5 cubic centimeter were used. A 2 per cent suspension by volume of sheep cells was used as indicator, and special effort was made to have it with as little variation as possible during the course of the work. Pooled sera of not less than four guinea pigs, obtained on the afternoon before the test, were used for complement. Both the hæmolysin and the complement were titrated on the morning before setting up the test proper, the latter in the presence of the antigen. Sera not more than three days old were inactivated at 56° C. for fifteen minutes, and those over three days old, for thirty minutes. The sera were used in amounts of 0.1 cubic centimeter.

In the ordinary technic both the primary and the secondary incubations were carried in a water bath at 37.5° C., while with the Kolmer technic the primary incubation was done in the ice box for eighteen hours followed by five minutes in the water bath. The secondary incubation was in the water bath for one hour.

The readings with both methods were made after the tubes had stood in the ice box from two to three hours, to allow partial settling of the unhæmolyzed cells. Readings were recorded according to the method of Citron,[14] in which complete absence of

[14] Kolmer, J. A., Infection; Immunity and Biologic Therapy, 3d ed. W. B. Saunders & Co., Philadelphia, page 472.

hæmolysis is recorded as 4-plus and, in the weaker reactions, 75 per cent hæmolysis as 1-plus and less than this as plus-minus. It is to be noted that for the present purpose even reactions of the last degree are considered positive, in full realization of their doubtful significance, at least in the diagnosis of treponematous infections.

CASES EXAMINED

The five hundred cases here dealt with were individually unselected except that, the findings in lepra reaction proving of particular interest, as many cases of this as could be obtained during the course of the work (82 cases) were included. According to the classification used in the Culion Leper Colony, about 70 per cent were of the mixed type, about 20 per cent cutaneous, and about 10 per cent neural. Most of them (434 cases) had been treated with chaulmoogra-oil derivatives for two years or more; sixty-six were untreated new arrivals; fifty-nine cases were on the negative list (that is, they were clinically arrested and bacteriologically negative), and several of these had practically completed the two-year observation period after having become negative.

The total group includes two series. The first comprises three hundred cases under treatment by one of us (E.R.-P.), in all of which a careful physical and historical examination was made. The sera of one-half of this group were tested by both methods. In the second series, of two hundred sera, the Kolmer method alone was used. As these cases were under treatment by other physicians, only those showing any degree of fixation were examined by us for evidence of a treponematous infection. Reactions made at the request of the attending physicians on account of suspected luetic or frambœsial infection are not included.

RESULTS

The results of the reactions are summarized in Table 1. Of the first group (all specially examined clinically), thirty-two cases were suspected of suffering from yaws, and of these twenty-six, or 81 per cent, gave positive reactions. Eighteen were suspected of being syphilitic, and fifteen, or 83 per cent, gave positive reactions. In two hundred fifty, no evidence of either syphilis or yaws could be determined; eighteen of these (7.2 per cent) gave reactions that were positive to some degree.

In the second group, of two hundred cases, under the treatment by other physicians, twenty-six gave positive reactions

and were examined clinically by us. In ten a diagnosis of yaws could be made, and seven were diagnosed as syphilitic. The remaining nine patients gave neither history nor clinical signs of a treponematous infection.

TABLE 1.—*Incidence of the Wassermann reaction in lepers with and without clinical evidence of treponematous infection.*

Case groups.	Number.	Percentage of group.	Wassermann reaction.		
			Negative.	Positive.	Percentage positive.
Group 1: [a]					
Apparently uncomplicated	250	83.3	232	18	7.2
Yaws suspected	32	10.6	6	26	81.2
Syphilis suspected	18	6.0	3	15	83.3
Total	300		241	59	19.7
Group 2: [b]					
Evidence of yaws	10		0	10	
Evidence of syphilis	7		0	7	
No evidence of either			174	9	
Total	200		174	26	13.0
Grand total	500		415	85	17.0

[a] All cases examined clinically for evidence of yaws or syphilis.

[b] Only cases giving positive Wassermann reaction were examined clinically for treponematous infection.

Taking both groups together (totaling five hundred cases), eighty-five cases, or 17 per cent of the total, gave reactions that were positive in some degree; but fifty-eight, or 68 per cent, of these were in cases of known or suspected treponematous infection. Only twenty-seven more or less strongly positive reactions were in patients not under such suspicion. These will be discussed in detail.

Yaws group.—Of the forty-two cases suspected or diagnosed as suffering from yaws, thirty-six gave positive Wassermann reactions. Data on these cases are given in Table 2.

In Tables 2, 3, 4, and 5 the following abbreviations are used:

K = Kolmer antigen and technic.
P. A. = Plain alcoholic extract of beef heart. Ordinary technic.
Ch. A. = Cholesterinized alcoholic extract of beef heart. Ordinary technic.
M = Mixed.
N = Neural.
C = Cutaneous.
Sl = Slight.

Mod = Moderate.
Mkd = Marked.
Arr = Arrested.
0 = Not done.

TABLE 2.—*Cases of leprosy with clinical evidence of yaws.*

No.	Age.	Leprosy.				Wassermann reaction.			Remarks.
		Type.	Stage.	Duration.	Bacteriological examination.	K	P. A.	Ch. A.	
	Yrs.			Yrs.					
1	10	M	Sl	2	+	4 +	0	0	Active lesions.
2	26	M	Mkd	15	+	3 +	0	0	
3	22	M	Mod	8	+	3 +	0	0	
4	17	M	Mkd	11	+	1 +	0	0	Treated.
5	19	M	Mod	11	+	4 +	0	0	
6	25	M	Mod	7	+	1 +	0	0	
7	17	M	Mkd	6	+	4 +	0	0	Lepra reaction.
8	24	N	Mkd	13	+	4 +	0	0	Active lesions.
9	59	M	Mod	4	+	4 +	0	0	
10	18	M	Mod	10	+	4 +	0	0	
11	30	M	Mod	3	+	3 +	0	0	
12	22	M	Mkd	7	+	4 +	0	0	
13	14	M	Mkd	6	+	4 +	0	0	
14	35	M	Mkd	9	+	4 +	0	0	
15	17	M	Mkd	8	+	4 +	0	0	Lepra reaction.
16	26	N	Mod	11	+	4 +	0	0	
17	23	M	Mod	12	+	1 +	0	0	Treated.
18	24	M	Mod	14	+	3 +	0	0	
19	35	M	Mkd	12	+	2 +	0	0	Lepra reaction.
20	17	M	Sl	10	+	4 +	0	0	Treated.
21	23	M	Mkd	7	+	4 +	0	0	
22	22	M	Mod	5	+	4 +	0	0	
23	20	C	Mod	5	+	—	—	1 +	Treated.
24	19	M	Sl	3	+	4 +	3 +	4 +	
25	18	M	Mkd	3	+	2 +	—	3 +	
26	20	M	Mod	3	+	2 +	2 +	4 +	
27	26	M	Mkd	2	+	4 +	4 +	4 +	
28	25	M	Mkd	2	+	4 +	4 +	4 +	
29	31	N	Arr	21	—	4 +	4 +	4 +	
30	33	N	Arr	18	—	2 +	±	2 +	
31	52	N	Arr	24	—	4 +	—	4 +	
32	45	M	Sl	10	+	4 +	1 +	4 +	
33	50	M	Mkd	13	+	4 +	4 +	4 +	
34	31	M	Mkd	2	+	3 +	1 +	4 +	
35	11	M	Mod	4	+	—	—	2 +	
36	25	M	Mkd	3	+	2 +	—	3 +	
37	32	M	Mkd	6	+	—	—	—	Tertiary, treated.
38	51	M	Mod	3	+	—	—	—	
39	35	M	Mod	3	+	—	—	—	
40	24	N	Arr	14	—	—	—	—	Treated.
41	20	C	Mod	9	+	—	—	—	
42	8	C	Mod	3	+	—	—	—	

Six of the patients placed in this group yielded negative Wassermann reactions, either because of error in diagnosis or because the infection had been overcome. Two of these had received arsphenamine treatment. Three others of this group had received such treatment but still yielded weakly positive reactions; evidently, not enough time had as yet elapsed for the reagin to disappear completely. A point of interest is that in six cases there was a slightly greater degree of fixation with the cholesterinized than with the Kolmer antigen (Nos. 23, 25, 26, 34, 35, and 36), while no serum in this table shows the converse in this respect.

Syphilis group.—Twenty-five cases were suspected of being syphilitic. Twenty-two of these gave positive Wassermann reactions, including one plus-minus reading. Data on these cases are given in Table 3.

TABLE 3.—*Cases of leprosy with clinical evidence of syphilis.*

No.	Age.	Leprosy.				Wassermann reaction.			Remarks.
		Type.	Stage.	Duration.	Bacteriological examination.	K	P. A.	Ch. A.	
	Yrs.			Yrs.					
1	43	N	Arr	6	—	2 +	0	0	
2	34	C	Sl	5	+	2 +	0	0	
3	28	C	Mod	9	+	4 +	0	0	
4	64	M	Mkd	4	+	2 +	0	0	
5	31	M	Mod	10	+	4 +	0	0	
6	27	N	Mkd	10	—	4 +	0	0	
7	38	M	Mkd	18	+	3 +	0	0	Lepra reaction.
8	28	M	Sl	3	+	2 +	0	0	
9	25	M	Mod	4	+	3 +	0	0	
10	55	M	Mkd	15	+	3 +	0	0	
11	35	M	Mkd	9	+	2 +	0	0	Lepra reaction.
12	51	M	Sl	4	+	4 +	1 +	2 +	
13	42	M	Mod	5	+	4 +	4 +	4 +	
14	47	M	Arr	?	—	4 +	4 +	4 +	
15	23	C	Mod	10	+	4 +	1 +	2 +	
16	25	M	Mkd	5	+	4 +	4 +	4 +	
17	20	M	Mod	3	+	3 +	—	2 +	
18	27	M	Mod	15	+	4 +	4 +	4 +	Treated.
19	24	M	Mod	3	+	1 +	1 +	1 +	
20	34	M	Mod	1	+	3 +	—	±	Treated.
21	45	M	Mod	2	+	4 +	2 +	3 +	Lepra reaction.
22	44	M	Mod	9	+	—	—	—	
23	48	M	Mod	10	+	—	—	—	Treated.
24	38	M	Mod	9	+	—	—	—	
25	36	M	Mod	3	+	—	—	—	

One case (No. 23) that was negative had previously received antisyphilitic treatment. Two cases (Nos. 18 and 20), although they had been treated, were still positive. It is to be noted that, where differences in the degree of reaction are seen in this group, the Kolmer technic gives the higher degree of fixation (Nos. 12, 15, 17, 20, and 21. This is in contrast with the findings in the yaws group and will be referred to again.

Apparently uncomplicated group.—Of particular interest in the present connection are the cases in which positive reactions were obtained but in which no clinical evidence of complication by syphilis or yaws could be elicited. The data are given in Tables 4 and 5. It must be remarked that, in spite of lack of such evidence, treponematous infection cannot be definitely ruled out. Some of these patients were very ignorant and could not answer questions intelligently. Moreover, four of them were Moros from northern Mindanao, a region in which yaws is known to be prevalent. It is to be noted that of eighteen unselected Moros tested seven, or 39 per cent, were positive, and most of them strongly so—a much higher rate than in the total group.

Of these twenty-seven cases, the majority (sixteen) are classed as lepra reaction cases. As this condition will be discussed in the next section, only the remaining eleven (41 per cent of the group) will be considered at this point. In eight of these the Wassermann reaction was strongly positive. In each of the three weak reactions but one antigen gave any degree of complement fixation; in two instances this was extremely weak, and in all of them the reactions were negative on retest, though the patients had received no antitreponematous treatment. It is significant that, in all three, the extent of the actual leprotic lesions was slight and the disease was not active; it seems incredible that even this slight degree of binding could be ascribed to it, for in view of the other results it is to be expected that only extensive active leprosy can give positive results. It is therefore felt that these reactions cannot be considered as affording evidence of positive results from leprosy in its ordinary phases.

The remaining eight were strongly positive. Three have died, primarily of nephritis; two of these had marked cutaneous leprosy. Five received antitreponematous treatment after the test and three became negative, including two of those that died; the third one has recently become weakly positive again. The

TABLE 4.—*Patients without evidence of leprosy or yaws.*

[Without lepra reaction.]

No.	Age.	Leprosy.				First test.			Antitreponematous treatment.	Retests.			Remarks.
		Type.	Stage.	Duration.	Lepra reaction.	K	P. A.	Ch. A.		K	P. A.	Ch. A.	
	Yrs.			*Yrs.*					*Injections.*				
1	65	M	Mkd	9	None	4 +	4 +	4 +	7	—	0	0	Died. Nephritis.
2	45	M	Mkd	3	do	4 +	4 +	4 +	6	—	0	0	Do.
3	23	M	Mod	2	do	4 +	4 +	4 +	10	— 2 +	0 —	0 ±	Insufficient treatment.
4	38	M	Mod	6	do	4 +	0	0	3	3 +	0	0	Do.
5	22	M	Mod	7	do	4 +	0	0	3	4 +	0	0	Do.
6	20	N	Neg	7	do	4 +	4 +	4 +	0	4 +	2 +	4 +	On "Negative List."
7	35	M	Mod	3	do	3 +	0	0	0	4 +	0	0	
8	60	M	Mkd	9	do	4 +	3 +	4 +	0	0	0	0	Died. Nephritis.
9	14	C	Sl	5	do	—	—	±	0	—	—	—	Original reaction doubtful.
10	14	M	Sl	1	do	—	—	2 +	0	—	—	—	Do.
11	30	M	Sl	18	do	±	0	0	0	—	—	—	Do.

other two had received but three injections when last tested and their reactions were still strong.

INFLUENCE OF LEPRA REACTION

Lepra reaction [15] is of particular interest to the clinician, and it is now being found to be so from the serological aspect. Because of this a disproportionately large number, totaling eighty-two, were tested. Twenty-three, or 28 per cent, gave positive findings. Of these but four were diagnosed clinically as syphilitic and three as frambœsial. (See Tables 2 and 3.) It would seem that such complications are not unusually frequent in lepra reactions seen here, so far as clinical evidence can show.

With further reference to the uncomplicated group giving positive Wassermann reaction (Table 5), it is striking that sixteen, or 58 per cent, were to be classed as lepra-reaction cases. In fourteen this condition actually existed at the time of the first test. The other two gave evidence of being in this general condition.[16] One had recently had it, and the other had it in the interval between the tests; in both the protein abnormality referred to persisted.

It is seen that of these sixteen cases only one (No. 16) gave a strong reaction (3-plus); this patient, a Moro, had been given three injections of neoarsphenamine when last retested and the reaction had become 4-plus.

The remaining fifteen cases gave weak reactions, and with at most two antigens when three were used. These results are markedly in contrast with those in the group of cases without lepra reaction discussed above. Retests were made on fourteen of these patients; the other one died of leprous cachexia due to the persistent reactions. Six were negative on retest, in four

[15] By lepra reaction we mean a rather ill-defined occasional phase in the course of leprosy, characterized by exacerbation of the old cutaneous lesions, with or without the appearance of new ones, such as macules, papules, and infiltrations that may later subside or remain as permanent lesions; there may or may not be fever, but this is usual in the more-marked degrees of the phenomenon. Other symptoms may accompany this reaction, such as malaise or neuritic or general muscular pains. The nature of this reaction is not well understood. While some consider that it is due to a temporary toxæmia, others are of the opinion that the disturbance is of anaphylactic nature. The actual exciting cause may be anything that lowers the resistance of the body, or treatment with the ethyl esters; the exciting factor is not always determinable.

[16] It may be stated here that work under way in the Culion laboratory shows that there is marked abnormality of the serum proteins in lepra reaction.

TABLE 5.—*Patients without evidence of syphilis or yaws.*

[With lepra reaction.]

No.	Age.	Leprosy.				First test.			Antitreponematous treatment.	Retests.			Remarks.
		Type.	Stage.	Duration.	Lepra reaction.	K	P. A.	Ch. A.		K	P. A.	Ch. A.	
	Yrs.			*Yrs.*					*Injections.*				
1	12	C	Mod	4	At time	±	0	0	0	—	0	0	Reaction subsided. Two retests.
2	28	M	Mod	10	do	1 +	—	1 +	0	—	0	0	Do.
3	26	M	Mkd	18	do	—	—	2 +	0	—	—	—	Do.
4	27	C	Mkd	8	do	1 +	±	2 +	0	—	—	—	Reaction subsided.
5	17	M	Mod	5	do	+	0	0	0	—	0	0	Reaction persists.
6	16	M	Mkd	9	do	1 +	0	0	0	—	0	0	Do.
7	48	C	Mod	6	do	1 +	—	2 +	0	—	—	—	Reaction subsided.
8	28	M	Mod	10	do	1 +	—	2 +	0	— ± ±	— — —	1 + 2 + ±	Reaction persists.
9	34	M	Mod	12	do	1 +	—	2 +	0	2 +	—	±	Reaction between tests; subsided.
10	25	M	Mod	13	Recent	1 +	0	0	0	—	—	2 +	Do.
11	23	M	Mkd	7	Later	—	2 +	3 +	0	±	—	2 +	Reaction between tests only; subsided.
12	13	C	Mod	6	At time	1 +	0	0	0	±	0	0	Reaction subsided.
13	29	M	Mkd	9	do	—	—	2 +	0	0	0	0	Died. Leprous cachexia.
14	59	C	Mkd	7	do	1 +	0	0	0	±	—	—	Reaction persists.
15	27	M	Mod	4	do	1 +	0	0	2	1 + —	0 —	0 —	Two injections neoarsphenamine. Reaction subsided between retests.
16	13	M	Mkd	5	do	3 +	0	0	3	4 +	0	0	Two injections neoarsphenamine before retest. Reaction subsided.

after subsidence of the lepra reaction, and in the other two in spite of persistence of this condition. Eight remained positive; in five there was no clinical evidence of lepra reaction at the time of retest, but three had had a reaction in the interval between the tests. In the other three the condition still persisted. One of the patients with persistent Wassermann after subsidence of the lepra reaction was given two injections of neoarsphenamine and became negative.

INFLUENCE OF TYPE OF LEPROSY

Those who believe that the Wassermann reaction is positive in leprosy find marked differences between the different types of the disease, and invariably report higher incidence in the cutaneous and the mixed types than in the neural. We have found no relation with the clinical type. Of three hundred two patients whose clinical classification was recorded, two hundred twenty-three were of the mixed type, forty-six were cutaneous, and thirty-three were neural, most of these the late, "burned out" variety. The percentages of positives in these groups were 19.7, 17.3, and 18.2, respectively. The slightly greater proportion in the mixed group is apparently due to the relatively greater number of lepra-reaction cases among them.

INFLUENCE OF ANTILEPROSY TREATMENT

As stated above, Goodpasture found some differences in the incidence of the positive reactions in the treated and the untreated cases. This has not been our experience. Of the total of five hundred cases, sixty-six were new arrivals that had not received any antileprosy treatment; eleven of these were positive. The remaining four hundred thirty-four either were under antileprosy treatment or had received it; seventy-four gave positive reactions. The percentages, 16.7 and 17, are practically identical.

CHILDREN

In the first series there were forty-six children under 15 years of age. Of these, five gave positive reactions, two of which (Nos. 9 and 10, Table 4) were probably of no significance, thus leaving three cases, or 6.5 per cent. This is much lower than the general average, and is of particular interest because in marked contrast with the findings of Lloyd, Muir, and Mitra,[17] who found it positive in 81 per cent of the children examined by them.

[17] Ind. Journ. Med. Res. 11 (1923) 1.

EFFECTS OF ANTITREPONEMATOUS TREATMENT

As the effect of antitreponematous treatment furnishes important evidence with regard to the causation of positive Wassermann reaction in leprosy, the data available are collected here. Thirty-two positive cases were treated with neoarsphenamine and the reaction repeated one or more times. The rest were not treated, for various reasons, the most important of which is that the treatment is not without danger in lepers as seen in the Culion colony. The results of the tests, together with data on the number of injections and the time interval from the last injection to the time of taking the blood, are given in Table 6.

TABLE 6.—*Cases of leprosy, Wassermann positive, treated with neoarsphenamine.*

No.	Clinical diagnosis.	Before treatment.	Injections.	Time interval from last injection.	After treatment.
1	Yaws	4 +	3	1 year	—
2	do	3 +	3	8 months	—
3	do	4 +	6	do	3 +
4	do	4 +	5	do	±
5	do	3 +	5	6 months	—
6	do	4 +	4	4 months	2 +
7[a]	do	4 +	6	3 months	4 +
8[b]	do	4 +	7	do	4 +
9	do	4 +	2	do	±
10	do	3 +	4	do	1 +
11	do	3 +	7	do	—
12	do	4 +	3	do	—
13	do	4 +	7	do	—
14[a]	do	4 +	8	2 months	3 +
15	do	2 +	5	do	—
16	do	1 +	4	do	—
17	Syphilis	4 +	5	6 months	—
18	do	2 +	8	4 months	—
19	do	2 +	4	do	—
20	do	4 +	8	do	—
21	do	4 +	4	3 months	—
22	do	4 +	2	do	—
23	do	4 +	4	do	1 +
24	do	2 +	6	2 months	—
25	do	3 +	3	do	—
26	do	4 +	3	do	—
27	do	4 +	3	do	—
28	do	2 +	3	6 days	—
29	?[c]	1 +	2	2 months	—
30	?[c]	4 +	7	do	—
31	?[c]	4 +	10	do	—
32	?[c]	4 +	6	4 months	—

[a] Active lesions of yaws healed as result of treatment.
[b] Chronic ulcerative yaws, healing.
[c] Gave neither history nor signs of syphilis or yaws.

Out of the thirty-two cases treated, the reaction was changed from positive to negative in twenty-three. In seven others it showed a greater or less diminution in strength; these were, with a single exception, cases of yaws, in which disease the reaction is known to disappear more slowly than in syphilis, as has been shown by Goodpasture and de Leon.[18] In only two cases did the strength of the reaction remain unchanged, but these cases were frank cases of yaws and the lesions have already disappeared.

During the first part of this work, as will be seen in Table 4, five cases that gave no history and showed no clinical evidences of syphilis or yaws gave complete fixation, with the three antigens used. However, syphilis could not be definitely excluded. Because of the degree of the reaction, we believe they were suffering from either syphilis or yaws. One case died before antitreponematous treatment was begun, and another was of a neural type and had been a negative leper for nearly two years. It is obvious that in this case the reaction could not have been due to the leprosy. In three cases antitreponematous treatment was given, and these cases gave negative results after a series of injections, which we consider a confirmation of our opinion as to the cause of the reaction in these cases.

DISCUSSION

It is evident from the findings presented that, if the Wassermann reaction is ever positive because of serum changes essential to leprosy itself, it certainly cannot be said to be so in the same sense that it is in syphilis and yaws. Clearly, it is usually negative and can have no diagnostic or prognostic significance. Most of the patients who gave positive Wassermann reaction also gave evidence, either physical or in their histories, of syphilis or yaws. Incidentally, the latter would appear to be considerably the more prevalent in the Culion Leper Colony. This is in conformity with the existing conditions in the Philippines; for, whereas syphilis is not common except in the larger cities, yaws is endemic and prevalent in many localities.

Analysis of the positive group without clinical evidence of syphilitic or frambœsial complication has convinced us that there are two reasons for such reactions; one is unrecognized syphilis

[18] Philip. Journ. Sci. 22 (1923) 221-231.

or yaws, the other lepra reaction. Of the eleven cases in the first group three gave very weak reactions that were probably of no significance, as they did not persist, and in these cases the leprosy was but slightly advanced. The remaining eight had strong reactions which we have come to believe due only to treponematous infection. The results of neosalvarsan treatment in certain of these cases tend to confirm this view, for that drug has little effect—at least, general—in leprosy, whereas it caused the disappearance or reduction of the Wassermann reaction in the cases treated.

Though most of the positive findings may thus be ascribed to treponematous infection, there remains a group of cases that seem to indicate that leprosy may of itself cause complement fixation in the Wassermann reaction, at least to some degree. All of these were in the condition known as lepra reaction. In patients presenting this phenomenon in a high degree there is marked clinical disturbance, necessitating hospitalization. Marked abnormality of the serum proteins is being found in work in progress in the Culion laboratory. Evidently there is some change, qualitative or quantitative, that tends to cause nonspecific binding of complement in the presence of lipoidal antigens. This has not occurred in most of the cases of lepra reaction as the tests have been carried out, and has not been strong when it has occurred in cases free from evidence or reasonable presumption of treponematous infection. Though this apparently nonspecific fixation of complement occurred, in one instance or another, with all the antigens used, it seems to be most frequent and strongest with the cholesterinized crude alcoholic. Why some cases give this fixation and others do not we are unable to say in the present state of our knowledge of leprosy, and particularly of this most interesting phase of the disease.

There remains, of course, the possibilty that an underlying latent treponematous infection exists in these cases, which is perhaps responsible for the lepra reaction. This seems very doubtful to us. In the first place, the fact that these positive reactions tend to disappear spontaneously with subsidence of the lepra reactions is evidence that they are dependent upon the changes in the balance between host and the infecting organism. Of the sixteen Wassermann-positive lepra-reaction cases without clinical evidence of treponematous complication, all but one gave weak Wassermann reactions. A treponematous

complication active enough to be responsible for the lepra reaction would be expected to give a strong Wassermann reaction (as in the other groups), at least in a fair proportion of cases. On the other hand, were these positive tests due to a "provocative" effect of the lepra reaction in latent treponematous infections, we would again expect a larger proportion of strong reactions than 1 out of 16 (considering here only the clinically uncomplicated group). It is true that one of these cases was given neosalvarsan and became negative, but it is not certain that he might not have become so had no such treatment been given.

Further evidence that leprosy itself has, with the exception noted, no influence on the reactions obtained is that the findings have no relation to type of case or to antileprosy treatment. Those who have reported the reaction as positive in this disease have almost invariably found the incidence higher in the cutaneous and the mixed cases than in the neural. This is not our experience, the figures for the three types being surprisingly similar. Nor does analysis of the data with reference to duration of the disease show any essential relation. Goodpasture's very interesting suggestion that antileprosy treatment may serve as a provocative measure could not be confirmed, for the percentages in the untreated and the treated groups were practically identical.

Again, the fact that the children gave relatively much fewer reactions is significant, for among them there were all degrees of the disease. We consider the lower rate due to the fact that they have had less opportunity for acquiring treponematous infection. It may be noted that yaws shows little, if any, tendency to become endemic at Culion, and the few acute cases that appear are treated.

A few observations with regard to the methods used may be recorded. Two cases (Nos. 9 and 10, Table 4) were negative with Kolmer's technic but showed fixation with the ordinary technic. This, coupled with differences of intensity of the fixation in cases of lepra reaction (Table 5), shows that the new method is distinctly less susceptible to slight nonspecific complement fixation than is the usual technic, particularly when an ordinary cholesterinized alcoholic extract is used as antigen.

In using the ordinary technic, we find that a very close titration of the cholesterinized extract for its antigenic unit is necessary. We have used two and a half antigenic units, which

gives a very slight excess of the antigen relative to the dose of both the complement and the hæmolysin. When the antigen was increased to one-third of the anticomplementary unit, a much larger percentage of fixations occurred—much larger than is the experience in nonlepers with this type of antigen. The tendency of the sera to fix complement in the presence of crude alcoholic extract of organs is evidently increased in leprosy.

Another point of interest, incidental to the main problem, is that, in those cases in which the positive reaction is due to yaws, the degree of fixation is apt to be greater with the cholesterinized alcoholic antigen than with Kolmer's. On the other hand, in those due to syphilis, Kolmer's antigen is apt to show a greater degree of fixation. So far as we are aware, this method has not been used in the study of yaws. While various authors have concluded that the Kolmer reaction is of superior practical specificity—as this term is used—in the serum diagnosis of syphilis, it is of interest that it should go so far as to give quantitative differences between the reagins of syphilis and of yaws. This seems to confirm the view of Castellani [19] that the reagins produced in the two diseases are different. Bowman [20] also came to the same conclusion, using plain alcoholic extract of guinea pig heart.

SUMMARY

The Wassermann reaction was made, using Kolmer's method, on the sera of five hundred lepers and, for comparison, about one hundred fifty of these were also tested by an ordinary method, using both plain and cholesterinized antigens. In the latter method it was found necessary to adjust the antigen dose closely, as an increase from the dose used, two and one-half antigenic units to four, resulted in an increased number of weakly positive or doubtful reactions.

The five hundred cases considered include sixty-six untreated new admissions, and cases that were under treatment at the time or that had been treated. Among the latter fifty-nine were negative and eighty-two were cases of lepra reaction.

Of the total group eighty-five, or 17 per cent, gave reactions that were positive in some degree, the doubtful plus-minus reactions being included. Of these, fifty-eight were in the group of sixty-seven cases that were under suspicion of yaws (42 cases) or syphilis (25 cases). In the remaining twenty-seven positive

[19] Journ. Hyg. Cambridge 7 (1907) 558.
[20] Philip. Journ. Sci. § B 5 (1910) 485–487.

cases neither history nor signs of yaws or syphilis could be elicited. Data on the clinically frambœsial and syphilitic groups are given.

The particularly interesting group without clinical evidence of treponematous infection is subdivided into those without and those with lepra reaction, eleven and sixteen cases, respectively. Of the former, eight gave strongly positive Wassermann reactions and of the latter but one. Further, the three weak reactions of the former group were in cases of slight leprous involvement, and the reactions were negative on retest. The one strong reaction in the latter group was in a Moro, particularly liable to yaws. These facts, and the results of antitreponematous treatment, strongly suggest that positive reactions were due to unrecognized syphilis or yaws.

The remaining fifteen weakly positive reactions in cases of lepra reaction were probably due to serum changes that occur in this condition, either peculiar to it or, more probably, particularly marked in it.

Our findings incidentally agree with those of others as to the sensitiveness of the Kolmer technic for the serum diagnosis of syphilis. Indeed, it seems to give less strongly positive reactions in yaws than does an ordinary cholesterinized antigen with the ordinary technic, thus affording further evidence that the reagins produced in these two diseases are not identical.

CONCLUSIONS

1. Our findings indicate that with Kolmer's and other refined methods the Wassermann reaction is negative in uncomplicated cases of leprosy in its ordinary phase.

2. Conversely, when the Wassermann reaction is clearly positive in cases of ordinary leprosy it has the same significance as in nonlepers.

3. In a certain proportion of cases of lepra reaction without evidence or presumption of treponematous complication weakly positive reactions are obtained. That such reactions are due to the same substance that is demonstrated in syphilis and yaws is highly improbable.

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STUDIES ON THE SEROLOGY OF LEPROSY, II

NITRIC ACID PRECIPITATION (BRUCK, MODIFIED)[1]

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INTRODUCTION

Investigations on the serology of leprosy have, until recently, been conducted along two lines. One involves attempts to develop a specific reaction with leprosy antigens; the other has to do with the relation of the Wassermann reaction to the disease.

The former problem is a most difficult one. The serology of infection with acid-fast bacteria in general involves peculiar difficulties, in that the antigens so far obtained have not permitted differentiation of members of the group. As regards leprosy there is, besides the close relation of the organism to that of tuberculosis, the handicap of the apparent non-cultivability of the causative agent, at least in its tissue form. A practical specific diagnostic test for leprosy seems not yet in sight.

The Wassermann reaction has not proved useful in this infection; for, though it has been generally understood that it is frequently positive, the reports have from the first been very discordant as regards the frequency. As the reaction has now been refined by syphilologists to increase its specificity or, rather, particularly for their purpose, positive reactions in leprosy uncomplicated by syphilis or yaws are at most infrequent. Mathis and Baujean,[2] using the technic of Calmette and Mossol, and recently Kolmer and Denney,[3] with the new technic of the former, find it regularly negative, as Yagley and Kolmer [4] have reported the Kahn precipitation reaction to be. Pineda,[5] applying

[1] Published with the approval of the Director of Health on recommendation of the Philippine Leprosy Research Board.

[2] Bull. Soc. Path. Exot. 8 (1915) 252.

[3] Arch. Dermat. & Syph. 8 (1923) 63.

[4] Arch. Dermat. & Syph. 8 (1923) 183–185.

[5] Antea, pp. 39–57.

Kolmer's technic in the Culion laboratory, has obtained results that agree with these reports in the main, though a small percentage of weakly positive reactions have been given by cases apparently free from syphilis or yaws, especially in "lepra reaction." It now seems clear that this question is of importance with reference to the treponematous infections rather than to the study of leprosy.

On the other hand, from the often reported occurrence of these—from the viewpoint of the syphilologist—false reactions in many cases of leprosy, particularly those with marked cutaneous involvement, it would seem that there is some peculiar serum change which, under some conditions, tends to bind complement in the Wassermann reaction. That the element involved is identical with the syphilitic reagin one would hardly suggest. It may be some factor or element irregularly present, or it may be something constantly present but only infrequently so to a sufficient degree to be demonstrable by this method.

That this general problem should be worked out need not be argued. There is urgent need of a test that will diagnose, or at least give presumptive evidence of, leprous infection in suspected cases, and also in contacts of known cases in order that by treatment latent infections may be prevented from evolving to the clinically positive stage. There is need of a test that may, by repeated application in cases under treatment, serve as a gauge of improvement. Finally, a test that would differentiate latency and actual cure in cases that have become clinically and bacteriologically "negative" would afford a far better basis of discharge than a fixed "negative period," which may be unnecessarily long in some cases and insufficient in others.

The problem is one that seems unlikely of ready solution, in spite of the advances that have been made. It would seem to call for much intensive work by a group of highly specialized investigators. From results obtained in the past it seems highly improbable that any established procedure or simple modification of such will suffice. It has, therefore, seemed profitable to approach the problem from another angle, to investigate certain of the physical and chemical peculiarities of the serum in leprosy.

The work to be reported in this and subsequent papers was begun along lines suggested by two recent reports. One is that

of Turkhud and Avari,[6] who found the formalin coagulation reaction, discovered by Gaté and Papacostas, to be positive in all cases of leprosy tested. The other is that of Schöbl and Basaca,[7] who found a distilled water globulin precipitation reaction, a modification of that of Klausner,[8] to be regularly positive. In line with these simple nonspecific reactions is the nitric acid precipitation test of Bruck, which has apparently not been applied in leprosy. The present report deals with the findings, with a slight modification, of this reaction in one hundred cases of leprosy.

THE NITRIC ACID REACTION

This reaction was described during the World War by Bruck[9] as possibly of value in diagnosing syphilis under conditions that would not permit the use of the Wassermann reaction. It is simple in principle, consisting of a rough determination of excessive (globulin) precipitate formed by nitric acid in dilute serum. It is not surprising that, as indicated by the several reports available to us, so nonspecific a reaction has met with disfavor as a means of diagnosing the presence of syphilitic infection.

Smith and Solomon[10] found disagreement with the Wassermann in 25 per cent of four hundred cases. In three hundred two nonsyphilitics 28 per cent gave doubtful or positive reactions. Stillians[11] had even poorer results, for in ninety-seven syphilitics in all stages there was 35 per cent disagreement with the Wassermann reaction. Of seventy-four nonsyphilitics 24 per cent gave positive reactions. Toyama and Kolmer[12] found that the reaction yielded 8 per cent false positives and was often negative when the Wassermann reaction was positive. Terada[13] found it to be somewhat less frequent than the Wassermann reaction in clinical syphilis (76 per cent of fifty-nine cases as compared

[6] Ind. Journ. Med. Res. 9 (1921–1922) 850.

[7] Philip. Journ. Sci. 25 (1924) 1.

[8] Wien. Klin. Wchnschr. (1908) 21, 214, 363, and Biochem. Ztschr. 47 (1912) 36 [cited by Kolmer, J. A., Infection, Immunity and Biologic Therapy. Philadelphia and London, 3d ed. (1923) 520].

[9] Münch. Med. Wochenschr. 64 (1917) 25 (cited).

[10] Boston Med. & Surg. Journ. 177 (1917) 321 (cited by Stillians and others).

[11] Journ. Am. Med. Assoc. 69 (1917) 2014.

[12] Journ. Cut. Dis. 36 (1918) 429.

[13] Kitasato Arch. Exp. Med. 3 (1919) 123.

with 86 per cent), and more frequent in nonsyphilitics (25 per cent of forty cases as compared with 12.5 per cent), but considers it of value when a more complicated test cannot be carried out.

Of interest in the present connection is the report of Corper and Fiala,[14] who tested the sera of two hundred five questionably or positively tuberculous and twenty-four nontuberculous persons. Of two hundred thirteen Wassermann-negative sera, one hundred thirteen (53 per cent) gave a positive Bruck reaction, most of them strong or fairly strong, a much higher percentage than obtained by others in nonsyphilitics. As for the relation to the stage of the tuberculous infection, it was more frequently positive among the moderately and far advanced (63 and 70 per cent, respectively) than among the nontuberculous (33 per cent), questionably tuberculous (46 per cent), and incipient (36 per cent) cases. No relation to the condition of the patient was to be seen. The authors could not see that the reaction gives any data of value.

A report by Mauchat, van Nitsen, and Walravens,[15] from tropical Africa, is also of interest. In thirty-two Europeans it was positive sixteen times; all of these were either syphilitic, with positive Wassermann reaction, or malarial or suspected malarial individuals. These positive reactions were all read as 1-plus. Of fifty-six Africans only two were negative, one with a phagedenic ulcer and the other with leprosy. In many the reaction was read as 2-plus, and in a few as 3-plus. They remark that the reaction is positive in syphilis and yaws when the Wassermann is positive, and generally in malaria though the Wassermann is negative. It is pointed out that practically all of the natives have chronic malaria.

TECHNIC

In the original technic 0.5 mil of serum is diluted with 2 mils of distilled water, and to this is carefully added 0.3 mil of a nitric acid solution of 1.149 specific gravity (approximately 25 per cent). In exactly ten minutes this is diluted with 16 mils of distilled water, and the tube is inverted three times to mix; ten minutes later the agitation is repeated. The test is read on the basis of the amount of undissolved precipitate, at the earliest a half hour later. As this is difficult to do before sedi-

[14] Am. Rev. Tuberc. 2 (1918–1919) 290.
[15] Compt. Rend. de la Soc. de Biol. 85 (1921) 720.

mentation has occurred, the tests are usually allowed to stand overnight.

At the beginning of this work efforts were made to make the test more precisely quantitative. However, it has become apparent that it has certain inherent weaknesses that, in view of the as yet indefinite significance of the results, do not recommend it for serious consideration; besides the differences in reaction of sera with similar globulin increases that follow from varying total protein contents, it is not free from technical error.

The formalin-coagulation reaction, though perhaps no more valuable or reliable, gives results that on the whole are similar, and it has the advantage of extreme simplicity and freedom from technical error. For this reason the technic used will be stated but briefly.

The sera were clear, or with at most but the faintest trace of hæmolysis, and were fresh and unheated. Some difficulty was met in determining the proper amount of acid solution, as the usual description of the standard solution is obviously incorrect. A solution was prepared [16] of which the standard quantity, determined with two known normal sera, was 0.25 mil. As each serum was tested by a titration series of four or five tubes, necessitating the use of one-half the usual amount of serum per tube, the acid solution was diluted one-half; this was found not to affect the results.

Three titration methods were tried, in which the variant was: (*a*) serum, (*b*) acid, or (*c*) final dilution. Of these, the first is probably the best, though the tests to be reported were done by the second. Better than either is a rough nephelometric determination of opacity. The sediments were examined after eighteen hours and read negative to 3-plus; the final record varied from negative to very strong, according to the number of tubes in the series showing precipitate and the amount of the precipitate.

FINDINGS

The results of the reaction with one hundred consecutive sera from lepers are given in Table 1 and, for comparison, those obtained with sixteen nonleprous controls. The cases were not selected as to type or extent of disease; as to complications, some of the sera were from hospital and clinic cases suspected of having syphilis or yaws.

[16] By Dr. G. A. Perkins, chief chemist of the Culion Leper Colony.

TABLE 1.—*Results of nitric acid precipitation reaction in sera of lepers and nonlepers.*

Case group.	Cases.	Degree of reaction.								
		Very strong.		Strong.		Moderate.		Weak.		Negative.
		Cases.	P. ct.	Cases.	P. ct.	Cases.	P. ct.	Cases.	P. ct.	
Untreated new cases	48	21	44	16	33	8	17	3	8	0
Treated clinic cases	41	3	7	20	49	14	34	4	10	0
Hospital cases *	11	3	27	8	73	0	.....	0	.....	0
Total	100	27	.....	44	.....	22	.....	7	.....	0
Negative lepers	10	1	.....	6	.....	1	.....	2	.....	0
Wassermann positive	21	8	38	11	52	1	5	1	5	0
Non-lepers:										
Professional staff	8	0	.....	0	.....	1	.....	4	.....	3
Laborers	8	0	.....	2	.....	4	.....	2	.....	0
Total	16	0	.....	2	.....	5	.....	6	.....	3

* Not suitable for antileprotic treatment.

Taking the lepers' specimens in total, seventy-one gave strong or very strong reactions, and twenty-nine were moderate or weak; none was negative. Considering the groups, there are decided differences in the distribution as regards degree of reaction. Of the newly arrived cases, not yet under antileprosy treatment, a relatively large number (44 per cent) gave very strong reactions, and only eleven (23 per cent) were moderate or weak. Of the cases under treatment only three (7 per cent) were in the very strong category, and eighteen (44 per cent) were moderately or weakly positive. The few reactions on lepers in the general hospital (not suitable for antileprosy treatment) were all strongly or very strongly positive.

Thirteen of the one hundred cases were on the "negative list;" that is, they showed no clinical signs of active leprosy and were bacteriologically negative. Such patients remain under observation and treatment for a further two years. The degree of reaction in the ten with negative Wassermann was fairly similar to that in the treated cases that are still positive. Evidently, the reaction does not tend to become negative rapidly in such patients.

The Wassermann reaction was performed (by Dr. E. V. Pineda) on all but two of the sera. In twenty-one inmates it was positive in some degree, apparently because of yaws or syphilis as a rule. Of these, all but two gave strong or very strong pre-

cipitation reactions; one, though from a probably syphilitic patient, with a 4-plus Kolmer Wassermann, was very weakly positive. On the whole, coincidence of these infections with leprosy apparently tends to increase the amount of precipitate, but this is so marked in most lepers that the difference is not great.

These results must be considered in comparison with the nonlepers. Comparatively few of these were available for examination. Of the eight specimens from the professional staff, only three were actually negative; four of the eight were weakly positive, and one was moderately strong. Of the eight laborers none was negative, and only two were weakly positive. In none of these nonlepers was the Wassermann reaction positive.

DISCUSSION

The results given by this reaction with the sera of nonlepers are of interest, because of the infrequency of negative findings. Even among the professional staff (most of them physicians), there was usually some excess of precipitate. As these individuals live in good circumstances, were apparently perfectly healthy and have remained so for nearly a year, one may doubt that a weakly positive reaction necessarily signifies the existence of a pathological condition. It would of course be difficult to determine clinically whether any particular individual is absolutely normal, but it may at least be said that if a positive reaction does depend upon an abnormality this may be very slight indeed.

It is not surprising that the laborers, whose grade of intelligence and mode of life are such as to make them more liable to infections of one kind or another, should give more frequent and stronger reactions. Still, the results seem excessive. It is to be remarked that the formol reaction has given decidedly fewer positive reactions in this nonleper group. I cannot ascribe this apparent oversensitiveness of the reaction to technic.

However this may be, it is evident that the reaction is strongly positive in the great majority of cases of leprosy uncomplicated by yaws or syphilis, indicating that there is as a rule a marked change in the serum in leprosy. The difference in the figures for treated and untreated cases are of some interest in the gross, indicating that treatment tends to reduce the abnormality on which this reaction depends. However, even in the negative cases tested such reduction had not gone very far.

Bruck [17] classes the significance of the reaction among those that demonstrate globulin excess. Whether this change is very slight in some cases, as indicated by the weak reactions, cannot be said, without data as to the total protein content of the sera. That the conditions may vary otherwise than quantitatively in different sera is indicated by certain other observations; it does not seem profitable to discuss these in detail.

The findings herein reported are, so far as leprosy work is concerned, of interest as a further indication that there is usually a decided protein abnormality in lepers' serum. Because of the findings in nonlepers, particularly those of the laboring class, it is doubtful whether or not the test would be of value in diagnosis of suspicious cases or contacts, unless a marked degree of serum change occurs very early. As for prognosis, it would seem from the results in the small group of negatives examined that the reaction does not decrease sufficiently in accord with symptoms, at least in patients under continued treatment, to serve as a gauge of clinical improvement. The frequent positive findings in nonlepers indicates that it cannot serve as a basis for discharge.

SUMMARY AND CONCLUSIONS

The problem of the development of serological methods for establishing the diagnosis, prognosis, or cure of leprosy demands attention. That there are decided serological changes in this condition is evidenced by frequent positive Wassermann reactions as this test is usually done, and by the recent findings of Turkhud and Avari with the Gaté and Papacostas formalin coagulation reaction and of Schöbl and Basaca with the distilled water globulin precipitation test. No satisfactory specific reaction has been developed, and this is particularly difficult of realization because of the noncultivability of the organism and its evidently close relation to that of tuberculosis, which infection would have to be differentiated. The Wassermann reaction, at least in the forms most suitable for the diagnosis of syphilis or yaws, is of little if any value in leprosy, except for the diagnosis of these complications.

A study of the serum of leprosy has been undertaken by means of certain nonspecific tests and by other means, in the hope of throwing more light on the changes occurring in this disease. Results with the nitric acid reaction of Bruck, somewhat modified as to technic, are here reported.

[17] Deutsche Med. Wchnschr. 48 (1922).

Of one hundred lepers' sera tested, ninety-three were read as moderately to very strongly positive, and none was negative. Antileprosy treatment apparently reduces the degree of reaction, it being very strong in a much smaller proportion (7 per cent) of the cases that have been under treatment than in new untreated cases (44 per cent). The results in ten cases clinically and bacteriologically negative were essentially the same as in the treated cases, the explanation for which fact is not apparent. The reactions in twenty-one sera giving some degree of the Wassermann reaction was essentially the same as in the untreated group.

Of a group of sixteen nonlepers, only three gave negative reactions, these among the professional staff, while two (laborers) were strongly positive. The frequency of weak reactions in apparently healthy individuals leads to speculation as to whether these weaker reactions are due to any abnormality at all.

That this reaction is of no value in the diagnosis of a particular disease is obvious; that it does not depend upon the presence of syphilis (or yaws) alone, and does not have the significance of the Wassermann reaction, is again shown.

NATURE AND AVAILABILITY OF THE PLANT-FOOD CONSTITUENTS OF PHILIPPINE GUANO

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The use of fertilizer by the sugar planters of the Philippines, which is becoming more and more general since the operation of sugar centrals, has resulted in the intensive and extensive working out of already known guano deposits of the country and in the search for new ones. Thousands of tons of guano, made up mainly of the excreta of bats, are being mined every year, the greater part of which is sold to local manufacturers of fertilizer who use this material mainly as a source of phosphoric acid for their brands of fertilizer; the remainder is disposed of to farmers who apply it directly to the soil. The quantity of guano purchased by three fertilizer concerns alone amounted in 1924 to approximately 6,000 tons, and the prospect is that the amount will increase gradually in the next few years, since many farmers are beginning to realize the importance of manuring in relation to better crop production. This ever-increasing demand for local guano renders it important, therefore, that its nature and the availability of its plant-food constituents be known; it was with this end in view that the present investigation was undertaken. However, no attempt has been made to ascertain the different forms in which the various constituents may exist in the manure.

This investigation was conducted on twelve samples, taken from more than a hundred which were fairly representative of large shipments coming from different regions of the Archipelago, and which were submitted for analysis to the Bureau of Science, during the latter half of 1923 and the first half of 1924. The samples were ground to pass a 30-mesh sieve and were tested for moisture (H_2O), organic and volatile matter other than water, total nitrogen, nitrogen liberated by direct distillation with alkali, nitrogen as nitrates, water-soluble and insoluble nitrogen, total phosphoric acid, water and citrate soluble phosphoric acid, total and water-soluble potash, calcium oxide, and iron and aluminium oxides. The samples were also tested qualitatively for magnesium and sulphates, and all were found to contain small amounts of those substances.

Unless otherwise stated, the samples were analyzed as received.

ANALYSIS OF THE PRINCIPAL CONSTITUENTS

One gram of each sample was first heated to constant weight in an oven at a temperature of from 100° to 105° C., to ascertain the amount of moisture. It was then ignited to constant weight to determine the organic matter. Finally the residue was treated with 20 cubic centimeters of 1:1 nitric acid and 5 cubic centimeters of 1:1 hydrochloric acid, the mixture was kept boiling for over an hour, and then diluted and filtered, receiving the filtrate and washing in a volumetric flask, which was filled to the mark. Aliquot parts were pipetted out and analyzed according to methods adopted by the Association of Official Agricultural Chemists.[1] The total nitrogen was determined by the Gunning method, modified to include the nitrogen of nitrates.

TABLE 1.—*Principal constituents of Philippine guano.*

[The figures in the last four columns were obtained by digestion with strong acid.]

Sample No.	Moisture (H_2O). 100°–105°C.	Organic matter.	Total nitrogen (N).	Phosphoric anhydride (P_2O_5).	Potash (K_2O).	Calcium oxide (CaO).	Iron and aluminium oxides (Fe_2O_3, Al_2O_3).
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
147366 [a]	33.85	34.90	4.69	4.20	0.43	13.30	10.63
150973 [b]	25.05	9.40	1.41	10.08	1.21	0.14	15.10
151016 [c] (71)	19.60	8.45	0.38	10.05	0.59	2.04	18.18
151016 [c] (72)	17.15	11.20	1.13	28.60	0.80	4.96	24.14
151025 [d]	30.40	18.45	1.22	11.78	0.77	7.86	7.78
151559 [e] (260)	21.23	11.27	1.07	6.51	1.10	1.02	11.73
151559 [f] (261)	29.20	9.45	0.97	7.44	1.02	0.73	6.94
151574 [g]	24.05	14.60	0.70	17.29	1.30	10.21	9.24
151676	16.60	19.40	2.38	5.94	0.34	1.46	11.27
151720 [h]	10.85	12.30	0.87	13.41	0.61	5.26	22.05
151749 [i]	7.38	7.65	0.94	20.69	0.95	1.90	18.49
151775 [j]	20.00	14.20	0.93	18.57	1.05	13 24	13.71

[a] Drawn from a shipment of 450 tons. The guano was obtained in Mataba, Masbate.

[b] Drawn from a shipment of 81.67 tons. The guano was obtained in Batangas.

[c] Drawn from a shipment of one carload. The guano was obtained in Iloilo.

[d] This guano was obtained in Marinduque.

[e] Drawn from a shipment of 95 tons. The guano was obtained in Batangas. Sample lost 15.59 per cent on air-drying.

[f] Drawn from a shipment of 24 tons. The guano was obtained in Batangas.

[g] Drawn from a shipment of 53 tons. The guano was obtained in Bohol.

[h] Drawn from a shipment of about 150 tons. The guano was obtained in Bohol.

[i] Drawn from a shipment of 40 tons. The guano was obtained in Iloilo.

[j] Drawn from a shipment of 417 tons. The guano was obtained in Masbate. Sample lost 18.16 per cent on air-drying.

[1] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Revised, Washington, D. C. (1920).

The results of the procedure are shown in Table 1. From these, as well as from the analyses of a vast number of samples drawn from large shipments and forwarded to the Bureau of Science from different parts of the country, it is evident that the average Philippine guano is mainly phosphatic guano, its nitrogen ranging from a fraction of 1 to 2 per cent, its phosphoric acid from 6 to 20 per cent, and its potash from a fraction of 1 to a little over 1 per cent. That it is phosphatic in character is just what would be expected, if the climatic conditions of the country are considered. Recent guano deposits are generally richer in nitrogen, as shown by the occasional analyses of samples coming from them. As time goes on, however, the heavy tropical rains penetrate the caves and leach out the soluble constituents of the deposit, among which figure principally nitrogenous substances, leaving behind the less soluble phosphatic and other less soluble compounds. The deposit stays moist and wet for a long time thereafter, particularly the bottom layers. This results in the further decomposition of nitrogenous substances and subsequent loss of nitrogen by the volatilization of the products of disintegration, namely, carbon dioxide and ammonia, and in the percolation of the compounds of the latter together with whatever nitrates were formed by the nitrifying agencies, and with the readily soluble phosphorus, potassium, and other compounds. In consequence, the deposit tends to become more and more phosphatic, and the phosphorus is transformed into forms which are insoluble in water and in ammonium citrate. This transformation is the more complete the larger the amount of the iron and aluminium compounds that have found their way into the mass from the slowly crumbling structure of the cave. The invasion of the water from the sea, near which many deposits are situated, produces a similar effect, as does also the access of atmospheric moisture and dew, although to a much less extent.

AVAILABLE NITROGEN

By available nitrogen is understood here the fraction of the total nitrogen liberated by direct distillation of the sample, or of the water extract of the sample with an alkali, and such other fraction which, in the presence of a reducing agent, is set free by an alkali.

The purpose in undertaking the study of the availability of nitrogen in guano was twofold; namely, (*a*) to ascertain the quantity of nitrogen obtainable by treating the material and its

water extract with an alkali, and the quantity of the same element present as nitrates; and (*b*) to learn the effect on the solubility of nitrogen of allowing the material to stand wet through varying lengths of time at room temperature and, incidentally, to find out whether or not under this condition there would be any indication that ammonification and nitrification were taking place.

NITROGEN BY DIRECT DISTILLATION

About 250 cubic centimeters of water and 20 cubic centimeters of concentrated sodium hydroxide solution were added to 5 grams of the sample, and the mixture was boiled to distill off the ammonia, which was caught in a measured volume of 0.1 normal solution of sulphuric acid. The excess of the acid was titrated back against 0.1 normal solution of sodium hydroxide, and the nitrogen calculated as usual.

For the determination of nitric nitrogen, the residue of the preceding distillation was cooled off, then diluted to 250 cubic centimeters, and then 3 grams of Davarda alloy were added. After the effervescence had subsided, the mixture was gradually heated to brisk boiling, the whole operation lasting about four hours. The distillate was, as before, caught in a known volume of 0.1 normal solution of sulphuric acid, the excess of which was titrated back against 0.1 normal solution of sodium hydroxide. The results are given in Table 2.

TABLE 2.—*Nitrogen determined by direct distillation of the sample.*[a]

Sample No.	Total nitrogen (N).	Nitrogen in the form of ammonia, ammonium salts, simple amids, soluble proteids, etc.	Ratio of nitrogen from NH_3, etc. to total.	Nitrogen in the form of nitrate.	Ratio of nitrogen from nitrates to total.
	Per cent.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
147366	4.69	0.88	18.76	1.37	29.21
150973	1.41	0.98	69.50	0.31	21.63
151016 (71)	0.38	0.09	23.63	0.08	21.05
151016 (72)	1.13	0.21	18.58	0.55	48.67
151025	1.22	0.22	18.03	0.32	26.23
151559 (260)	1.07	0.21	19.63	0.16	14.95
151559 (261)	0.97	0.41	42.27	0.17	17.52
151574	0.70	0.16	22.86	0.08	11.43
151676	2.38	0.19	7.98	0.24	10.08
151720	0.87	0.27	31.03	0.33	37.93
151749	0.94	0.77	81.91	0.03	3.19
151775	0.93	0.15	16.13	0.45	47.31

[a] The quantitative determination of nitrate nitrogen was preceded by a qualitative test with brucine, which test proved that all the samples contain this form of nitrogen.

WATER-SOLUBLE NITROGEN

Three 5-gram portions of each sample were placed separately in low, wide-mouthed bottles, and allowed to stand wet at room temperature for ten, twenty, and thirty days, respectively. The bottles were kept as far removed as possible from the ammonia of the laboratory and were covered with only a sheet of stiff paper in order to permit the free access of air. At the end of each of the periods the sample was transferred to filter paper and washed until the combined volume of the filtrate and the washing was 150 cubic centimeters. This quantity was diluted to about 250 cubic centimeters, and the nitrogen in it determined as above described.

TABLE 3.—*Water-soluble nitrogen dissolved by allowing the guano samples to stand wet for ten, twenty, and thirty days.*

Sample No.	Nitrogen (N) from ammonia, ammonium salts, simple amids, soluble proteids, etc.			Nitrogen (N) from nitrates.			Ratio of soluble nitrogen to total nitrogen.
	10 days.	20 days.	30 days.	10 days.	20 days.	30 days.	
	P. ct.	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.* [a]
147866	0.51	0.54	0.56	1.41	1.30	1.34	40.95
150973	0.23	0.23	0.24	0.36	0.34	0.34	41.84
151016 (71)	0.02	0.01	0.01	0.06	0.08	0.08	21.05
151016 (72)	0.04	0.06	0.06	0.24	0.55	0.31	24.86
151025	0.05	0.05	0.05	0.31	0.28	0.29	29.50
151559 (260)	0.04	0.03	0.04	0.17	0.16	0.17	19.62
151559 (261)	0.06	0.06	0.06	0.20	0.18	0.17	26.80
151574	0.01	0.01	0.01	0.11	0.09	0.08	17.14
151676	0.04	0.03	0.04	0.21	0.20	0.20	10.50
151720	0.04	0.04	0.06	0.36	0.36	0.36	45.98
151749	0.58	0.57	0.53	0.02	0.02	0.02	63.63
151775	0.04	0.03	0.03	0.47	0.47	0.45	54.84

[a] The solubility for the ten-day period was used.

The figures in Table 3 bring out prominently the following facts: (*a*) The comparatively large amounts of nitrogen in water-soluble form; (*b*) the relatively large amounts of nitrogen (3 to 48 per cent of the total) in the nitrate form, which shows plainly the degree of nitrification that the products of fermentation or hydrolysis of the proteids and simple amids of the guano has undergone; and (*c*) the no less inconsiderable amounts of substances present which are soluble in water and which upon treatment with an alkali yield ammonia. These substances are easily available to plants, regardless of whether they are

organic or inorganic ammonium salts, simple amids, urea, guanidine, etc., and also regardless of whether they are directly absorbed by the plants [2] or have first to go through the process of ammonification or nitrification before they can be utilized as nutrient.

Comparing the quantities of nitrogen shown in Table 3 that might exist in the samples as ammonium salts, urea, guanidine, and other soluble simple amids and soluble proteids with the corresponding quantities of nitrogen shown in Table 2, it is seen that the former are only small fractions of the latter, the differences probably being due to the nitrogen occurring as proteids insoluble in water but decomposable into ammonia, amino acids, and other organic compounds by direct treatment with an alkali.

However, the existence of a considerable portion of nitrogen in the nitrate form is the important characteristic which distinguishes Philippine guanos from guanos found in other parts of the world (for instance, Chile and Peru) where the climate is warm and dry. In the guanos from countries with a warm, dry climate the proportion of nitrate nitrogen to total nitrogen is very small [3] because, owing to the poor supply of moisture, the process of fermentation and the process of nitrification can take place only very slowly.

As to the effect that wetting the material continuously through varying lengths of time has on the solubility of nitrogen, the figures in Table 3, taken in general, show that, under laboratory conditions, no increase could by this means be secured in the amount of water-soluble nitrogenous compounds yielding ammonia upon treatment with an alkali.

Despite the seeming inconsistency of the figures in the table, which inconsistency is attributable only to the defect inherent in the method of determination (for all the necessary precautions were taken to insure accuracy of results), they nevertheless apparently indicate that no ammonification or nitrification had taken place during the whole period of the experiment; therefore, it would seem that ammonifying and nitrifying bacteria were either absent or became extinct during the period of storage of the samples.

[2] U. S. Bur. Agr. Bull. 158 (1914) 20.

[3] Hall, Fertilizers and Manures. E. P. Dutton & Co., New York, 2d ed. (1921) 233.

AVAILABLE PHOSPHORIC ACID

The study of the availability of phosphorus in guano has involved a test of the solubility of the phosphoric acid content of the guano in neutral ammonium citrate, and also a test of its solubility in water, as this solubility is affected by the length of time during which the sample has been kept wet.

CITRATE-SOLUBLE PHOSPHORUS

The citrate-soluble phosphoric acid was determined by the method adopted by the Association of Official Agricultural Chemists, except that the water-soluble portion of phosphoric acid was extracted in a manner similar to that recommended by Caro and Larison,[4] which consisted in adding about 200 cubic centimeters of water to 2 grams of the sample, and allowing the mixture to stand for one and one-half hours and then filtering. The results are given in Table 4.

TABLE 4.—*Citrate-soluble phosphoric acid calculated as phosphoric anhydride* (P_2O_5).

Sample No.	Citrate soluble.	Ratio of citrate soluble to total.	Sample No.	Citrate soluble.	Ratio of citrate soluble to total.
	Per cent.	*Per cent.*		*Per cent.*	*Per cent.*
147366	3.06	72.85	151559 (261)	0.00	0.00
150973	0.70	6.94	151574	3.96	22.90
151016 (71)	2.74	27.26	151676	2.08	35.00
151016 (72)	0.08	0.28	151720	3.57	26.62
151025	1.79	15.19	151749	1.93	9.32
151559 (260)	0.00	0.00	151775	3.65	19.66

WATER-SOLUBLE PHOSPHORUS

Samples weighing 2 grams each were placed in wide-mouthed bottles and allowed to stand wet for two, five, nine, sixteen, and thirty days. At the end of each of these periods the phosphoric acid of each sample which passed into solution was extracted with 25 cubic centimeters of water (in 5-cubic-centimeter portions), and the liquid decanted through a filter. The residue in the filter was washed with 25 cubic centimeters of water in about 5-cubic-centimeter portions, and this washing added to the filtrate. The filter was then torn into small pieces and returned to the bottle. The phosphoric acid in the filtrate was determined as previously described for total phosphorus. The

[4] Ind. & Eng. Chem. 17 (1925) 261.

content of the bottle was again allowed to stand constantly wet, until the next period, and the phosphoric acid that passed into solution was extracted and determined as before. This process was repeated for all the remaining periods. The results are given in Table 5.

In testing for the solubility of phosphorus in water care was taken that the supply of the liquid should be the same throughout the experiment; it was never more than the quantity necessary to keep the samples constantly wet. It has been shown that in difficultly soluble phosphates the amount of phosphorus that passes into solution is in direct ratio to the quantity of the solvent used, and it was to forestall any error that might arise from the lack of uniformity in the quantity of water employed that this precaution was taken.

TABLE 5.—*Phosphorus dissolved by keeping the guano samples wet through different periods of time.*

[The figures represent percentages of phosphoric anhydride.]

Sample No.	Number of days.					Total dissolved in thirty days.	Ratio of total dissolved in thirty days to total.
	2	5	9 [a]	16	30		
147366	0.63	0.38	0.16	0.27	0.24	1.68	40.00
150973	0.03	0.03	0.05	0.06	0.08	0.25	2.48
151016 (71)	0.06	0.04	0.03	[b] 0.07	0.06	0.26	2.58
151016 (72)	0.31	0.12	0.07	0.21	0.18	0.89	3.11
151025	0.19	0.16	0.10	0.13	0.13	0.71	6.02
151559 (260)	0.02	0.01	0.02	0.04	0.04	0.13	1.99
151559 (261)	0.03	0.02	0.03	0.07	0.06	0.21	2.82
151574	0.19	0.16	0.06	0.09	0.13	0.63	3.64
151676	0.02	0.03	0.03	0.03	0.04	0.15	2.52
151720	0.05	0.04	0.04	[b] 0.05	0.07	0.24	1.86
151749	0.08	0.03	0.03	[b] 0.03	0.06	0.23	1.11
151775	0.25	0.18	0.09	0.28	0.36	1.16	6.24

[a] Extracted with 50 cubic centimeters of water in 10-cubic-centimeter portions. The residues were washed twice only. Sample 151016 (72) was extracted with 50 cubic centimeters in 25-cubic-centimeter portions.

[b] The residue on the filter was washed with 50 cubic centimeters in two portions.

The low availability of phosphorus in Philippine guanos, as demonstrated in Tables 4 and 5, taken together with the high percentages of the oxides of iron and aluminium shown in Table 1, is an indication that the element exists mainly as phosphates of those two metals. However, this is not surprising because, as above stated, the humid climate and the recurrent torrential rains that prevail in this country are the main agencies by which local guanos become impoverished of their prin-

cipal plant nutrients. Although the soluble phosphorus is not easily lost by leaching, it is nevertheless converted into insoluble forms, such as tricalcium phosphate and the phosphates of the metals aforementioned.

Coming now to the relation of the length of time the sample has been kept wet to the amount of phosphorus that passes into solution, the figures in Table 5, though they fail to indicate any such relationship, on the other hand, do point out the following facts: (*a*) That the water solubility of phosphorus in Philippine guanos is, in general, very small for a given period of time; (*b*) that the dissolving process of phosphorus is gradual and extends over a long period of time; and (*c*) that the amount of phosphorus that passes into solution is not in proportion to the organic-matter content of the samples, nor do the partial amounts dissolved bear any definite relation to the period of wetting.

This lack of relationship between the organic-matter content of the material and the amount of phosphorus being dissolved may perhaps be attributed to the possible absence of salts,[5] which in solution would hydrolize and, especially, to the great preponderance of the difficultly soluble phosphates of iron and aluminium that were formed during the leaching process that the deposit underwent in nature. Examination of columns 3, 7, and 8 of Table 1, in connection with column 7 of Table 5, seems to elicit the fact that those samples gave up comparatively good quantities of dissolved phosphorus which contain relatively small amounts of iron and aluminium but relatively large amounts of calcium and organic matter.

Whether guanos of the kind here studied would, when placed in the soil, readily give up their phosphoric acid, thus either changing the relation of the soil to the plant[6] or enabling the plant to use the phosphoric acid directly, is a question the answer to which is beyond the scope of this article; but there is abundant evidence, from practical use, to show that the application of such guanos is highly beneficial to plants.

AVAILABLE POTASH

To 2 grams of each guano sample, placed in a beaker, 200 cubic centimeters of water were added, and the mixture was allowed to stand for one and one-half hours and then filtered.

[5] U. S. Bureau of Soils Bull. 41 (1907) 50.
[6] U. S. Bureau of Soils Bull. 48 (1908) 8.

The potash in the filtrate and washings was then determined by the platinic chloride method, as adopted by the Association of Official Agricultural Chemists.[7] The results are given in Table 6.

TABLE 6.—*Water-soluble potash (K_2O) in Philippine guanos.*

Sample No.	Total.	Water soluble.	Ratio of water soluble to total.	Sample No.	Total.	Water soluble.	Ratio of water soluble to total.
	Per cent.	*Per cent.*	*Per cent.*		*Per cent.*	*Per cent.*	*Per cent.*
147366	0.43	0.26	60.46	151559 (261)	1.02	0.15	14.70
150973	1.21	0.16	13.22	151574	1.30	0.03	2.30
151016 (71)	0.59	0.03	5.08	151676	0.34	0.09	26.47
151016 (72)	0.80	0.03	3.75	151720	0.61	0.07	11.47
151025	0.77	0.53	68.83	151749	0.95	0.57	60.00
151559 (260)	1.10	0.13	11.81	151775	1.05	0.11	10.47

The wide range of solubility in water of the potash in Philippine guanos, as shown by the figures in Table 6, indicates that potassium exists in different forms in the different masses; and since, in the case of plant nutrients, solubility and availability are identical, the inference is that no general conclusion can be established concerning the degree of availability of potash in local guanos. This statement is also true with reference to nitrogen and phosphorus.

SUMMARY

1. Philippine guanos are, in general, phosphatic guanos, the phosphorus existing mainly as phosphates of iron and aluminium. In freshly deposited guanos, however, the phosphorus exists mostly in immediately available form.

2. The water solubility of nitrogen in Philippine guanos—that is, the immediately available nitrogen—ranges from 16 to 60 per cent of the total, of which from 3 to 15 per cent may be considered as derived from ammonia, ammonium salts, simple amids, soluble proteids, etc., and the remainder from nitrates. An important characteristic of Philippine guanos is, therefore, the relatively large proportion of nitrogen that exists as nitrates.

3. Potash is invariably present in Philippine guanos in quantities generally not exceeding 1.5 per cent. From 3 to 68 per cent of this is immediately available to plants.

[7] Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists, Revised, Washington, D. C. (1920).

ADDITIONS TO OUR KNOWLEDGE OF THE BORNEAN FLORA, II

By ELMER D. MERRILL

Of the University of California, Berkeley

The first paper of this series was published in 1922;[1] like its predecessor, the present paper contains descriptions of presumably previously undescribed plants and the records of a few species hitherto not definitely known to occur in Borneo; among the latter is a representative of the genus *Harrisonia*, a genus previously unknown from Borneo. Nine species in the genera *Castanopsis*, *Coelodepas*, *Canarium*, *Pentace*, *Grewia*, *Kayea*, *Ardisia*, and *Callicarpa* are herein described as new. The material on which this paper is based was chiefly supplied by Mr. D. D. Wood, in charge of the Forestry Service of the British North Borneo Government. The actual types of the new species herein described are deposited in the herbarium of the University of California and, so far as duplicate material is available, isotypes will be sent to several of the larger herbaria, including that of the Bureau of Science.

FAGACEÆ

Genus CASTANOPSIS Spach

Castanopsis pearsonii sp. nov.

Arbor circiter 25 m alta, ramis ramulisque glabris, teretibus, lenticillatis, ramulis circiter 3 mm diametro; foliis coriaceis, oblongo-ellipticis, integerrimis, 15 ad 20 cm longis, 6 ad 8 cm latis, perspicue acuminatis, basi late acutis, supra glabris, olivaceis, nitidis, subtus pallidioribus et minute furfuraceo-pubescentibus, nervis primariis utrinque circiter 12, curvatis, arcuato-anastomosantibus, subtus perspicuis; petiolo glabro, 1 ad 1.8 cm longo; floribus ignotis; infructescentiis spicatis, pedunculatis, circiter 20 cm longis, pedunculo elongato, glabro, lenticellato, circiter 4 mm diametro; involucris paucis, ut videtur plerumque 1 vel 2, subglobosis, circiter 5 cm longis et 6 cm diametro, crassis, intus densissime fulvo-hirsutis, extus dense

[1] Philip. Journ. Sci. 21 (1922) 515–534.

cinereo-pubescentibus, spinis numerosis crassis pubescentibus fasciculatis rigidis curvatis 4 ad 8 mm longis instructis, fasciculis in lineis subobliquis dispositis; glans plerumque 3, triangulari-ovoideis, acutis, 3-angulatis, castaneis, circiter 2.5 cm longis et latis, decidue fulvo-pilosis, basi solummodo affixis.

BRITISH NORTH BORNEO, Rayoh, *1151 D. D. Wood, col. Evangelista*, January 25, 1924, on forested ridges, altitude about 225 meters.

This species, dedicated to former Governor A. C. Pearson, C. M. G., of British North Borneo, is strongly characterized by its large, subglobose involucres which are divided vertically by four straight, spine-free sutures, the fascicles of stout, curved, glabrous-tipped spines being arranged in somewhat irregular, oblique lines, the spaces between the rows of spines being about as wide as the basal parts of the fascicles, the spines not concealing the entire surface of the involucres.

EUPHORBIACEÆ

Genus COELODEPAS Hasskarl

Coelodepas brevipes sp. nov.

Frutex inflorescentiis petiolisque exceptis glaber vel ramulis ultimis parcissime pubescentibus, ramis teretibus, tenuibus, pallidis; foliis brevissime petiolatis, chartaceis, in siccitate olivaceo-viridis, nitidis, subtus pallidioribus, oblongis ad oblongo-lanceolatis, 12 ad 18 cm longis, 4.5 ad 6 cm latis, basi obtusis ad rotundatis, subtus utrinque 1- ad 4-maculari-glandulosis, apice perspicue longe acute acuminatis, margine distanter crenatis, nervis primariis utrinque circiter 10, perspicuis, arcuato-anastomosantibus; petiolo 2 ad 3 mm longo, minute denseque tomentoso; stipulis lanceolatis, rigidis, acuminatis, 5 mm longis; inflorescentiis plerumque axillaribus, solitariis vel fasciculatis, minute subferrugineo-pubescentibus, 5 ad 7 mm longis, glomerulis ♂ 5- ad 10-floris, distantibus, sessilibus, 4 mm diametro; calycis leviter pubescentibus, lobis 3, ovatis, acutis, circiter 1 mm longis; staminibus 6, filamentis deorsum (1.5 mm) connatis, partibus liberis 0.5 mm longis; ovarii rudimentum bifidum; floribus ♀ paucis ad basin spicarum ♂, sessilibus, circiter 5 mm longis; sepalis circiter 9, lanceolatis ad oblongo-lanceolatis, acuminatis, 1 ad 2 mm longis, utrinque dense pubescentibus; ovarium 3-loculare, tomentosum, stylis flabellato-incisis.

BRITISH NORTH BORNEO, Kalumpang, *1291 D. D. Wood*, July 25, 1924, in forests at low altitudes.

A species manifestly allied to *Coelodepas wallichianum* Benth. of the Malay Peninsula, but differing in its thinner, differently shaped, slenderly and acutely acuminate leaves, shorter petioles, deeply lobed pistillate calyces and other characters. It is the second species of the genus to be recorded from Borneo. The pistillate flowers are few in number, solitary or in pairs at the base of some staminate inflorescences.

Genus **HOMONOIA** Loureiro

Homonoia riparia Lour.

Homonoia riparia LOUR., Fl. Cochinch. (1790) 637; PAX & HOFFM. in Engl. Pflanzenreich 68[1] (1917) 114, *fig. 27.*

BRITISH NORTH BORNEO, Marudu Bay, *Mrs. E. Bateson 59,* June, 1923.

Curiously, this very widely distributed species has not hitherto been recorded from Borneo. It extends from India to Formosa through Malaysia to Timor and Celebes, always growing on the banks or in the beds of swiftly running streams.

BURSERACEÆ

Genus **CANARIUM** Linnæus

Canarium megalanthum sp. nov. § *Crassipyrena.*

Arbor circiter 15 m alta, perspicue ferrugineo-tomentosa, ramulis circiter 6 mm diametro; foliis circiter 40 cm longis, petiolis ferrugineo-tomentosis; foliolis plerumque 9, oblongis, chartaceis vel subcoriaceis, integris, in siccitate castaneis vel castaneo-olivaceis, subtus brunneis, breviter acuminatis, basi late rotundatis, plerumque inaequilateralibus, supra ad costa ferrugineo-tomentosis, subtus plus minusve ferrugineo-tomentosis; nervis lateralibus 13 ad 15, perspicuis, patulis vel leviter adscendentibus, circiter margine arcuato-anastomosantibus, reticulis subtus elevatis; petiolulis 4 ad 7 mm longis; inflorescentiis terminalibus, paniculatis, densissime ferrugineo-tomentosis, foliis subaequantibus, ramis paucis, inferioribus usque ad 9 cm longis; floribus longe pedicellatis, permagnis, circiter 1.5 cm longis, pedicellis crassis, usque ad 2 cm longis, bracteis ovatis ad oblongo-ovatis, crasse coriaceis, obtusis, utrinque dense tomentosis, circiter 1.5 cm longis et 1 cm latis; calycis lobis 3, crasse coriaceis, tomentosis, triangulari-ovatis, acutis, usque ad 1 cm longis; petalis 3, crassissime coriaceis, utrinque dense tomentosis, carinatis, orbicularis ad orbiculari-ovatis, obtusis, circiter 12 mm diametro; discus crassus, glaber, crasse coriaceus, circiter 7 mm diametro,

2.5 ad 3 mm altus; staminibus 6 in margine discum insertis, filamentis glabris, 3 mm longis, antheris leviter pubescentibus, anguste oblongis, filamentis subaequantibus; ovarium trigono-ovoideum, dense pubescens, 6 mm diametro; stigma capitatum, globosum, 3 mm diametro, longitudinaliter 3-sulcatum.

BRITISH NORTH BORNEO, near Weston, *No. 1213 D. D. Wood, col. P. Orolfo*, March, 1924, near the railroad line at low altitudes.

A species remarkable for its unusually large flowers, readily recognizable by this one character as well as by its dense ferruginous indumentum, few-flowered panicles, large bracts, and long stout pedicels. If properly placed within the section *Crassipyrena*, as the species are arranged by Engler, it comes nearest *Canarium balansae* Engl. but is remote from this particular species as it merely has in common with the latter the long-pedicelled flowers.

SIMARUBACEÆ

Genus **HARRISONIA** R. Brown

Harrisonia perforata (Blanco) Merr.

Harrisonia perforata (Blanco) MERR., in Philip. Journ. Sci. 7 (1912) Bot. 236; Enum. Philip. Fl. Pl. 2 (1923) 346.

BRITISH NORTH BORNEO, Lahad Datu, *Mrs. E. Bateson 33*, June, 1923.

Burma to southern China, the Philippines, and Java. The first representative of the genus to be recorded from Borneo; more commonly known as *Harrisonia bennettii* Hook. f.

TILIACEÆ

Genus **PENTACE** Hasskarl

Pentace laxiflora sp. nov.

Arbor circiter 8 m alta, ramis glabris, in siccitate rubro-brunneis, teretibus, ramulis circiter 2 mm diametro, dense cinereo-stellato-puberulis; foliis chartaceis ad subcoriaceis, oblongo-ovatis ad elliptico-ovatis, 6 ad 10 cm longis, 3 ad 4.5 cm latis, breviter acuminatis, basi plerumque rotundatis, tenuiter breviterque 3-nerviis, utrinque dense cinereo-stellato-puberulis, subtus pallidioribus; nervis primariis utrinque 4 vel 5, tenuibus, subtus distinctis, curvato-adscendentibus; petiolo puberulo, circiter 1 cm longo; paniculis dense cinereo-puberulis, laxis, usque ad 15 cm longis, ramis primariis patulis, inferioribus circiter 6 cm longis; floribus circiter 5 mm longis, pedicellis tenuibus, 2 ad 5 mm longis; calycis 3 mm longis, extus dense cinereo-puberulis,

lobis tubo aequantibus, ovatis ad oblongo-ovatis, acutis; petalis 5, spatulatis ad oblanceolatis, obtusis, 5 mm longis, glabris; staminoideis oblongis, 1.5 mm longis, crassis, pubescentibus; staminibus circiter 40, filamentis glabris, filiformibus, 3 ad 4 mm longis, plus minusve pantadelphis; ovarium oblongo-ovatum, pubescente, 3 mm longum, sulcatum, 5-loculare.

BRITISH NORTH BORNEO, Bundu, *No. 1804 D. D. Wood,* June 25, 1924, on slopes, with the Dusun name *takalis.*

A species apparently resembling *Pentace hookeriana* King which, however, has glabrous branchlets and leaves, much fewer stamens, and orbicular staminodes. It differs radically from *Pentace borneensis* Pierre in its fewer-nerved, glabrous leaves which have acute bases. Pierre's species was overlooked by me in preparing the manuscript of my Enumeration of Bornean Plants and is recorded below.

Pentace borneensis Pierre.

Pentace borneensis PIERRE, Fl. Forest. Cochinch. 2 (1888) sub *t. 151.*

SARAWAK, *Beccari 1261, 2063.* Known only from Borneo; the description is very imperfect.

Genus **GREWIA** Linnæus

Grewia pearsonii sp. nov.

Frutex erectus, 2 ad 3 m altus, partibus junioribus inflorescentiisque perspicue longissime ciliato-hirsutis; ramulis ultimis circiter 3 mm diametro, densissime breviter griseo-pubescentibus et pilis longis tenuibus patulis ferentibus; foliis lanceolatis ad oblongo-lanceolatis, chartaceis, 20 ad 35 cm longis, 5 ad 8 cm latis, integris, longissime tenuiter caudato-acuminatis, basi abrupte rotundatis, cordatis, 2 ad 4 cm latis, leviter inaequilateralibus, breviter 3-nerviis, supra in siccitate viridibus vel pallidis, subnitidis, costa perspicue hirsuta excepta glabris, subtus ad costa plus minusve pubescentibus et perspicue longe ciliato-hirsutis, nervis lateralibus parce ciliatis; nervis primariis utrinque circiter 12, subtus perspicuis, distantibus, curvatis, arcuato-anastomosantibus, reticulis primariis laxis, perspicuis; petiolo 6 ad 10 mm longo, dense pubescenti et perspicue ciliato-hirsuto; stipulis lineari-lanceolatis, circiter 2 cm longis, ciliatis; inflorescentiis paniculatis, terminalibus, circiter 10 cm longis, ramis paucis, ad 4 cm longis, omnibus partibus densissime breviter pubescentibus et longe ciliato-hirsutis; bracteis lanceolatis, acuminatis, longe ciliatis, usque ad 12 mm longis; bracteolis exte-

rioribus (3) ovatis, dense ciliatis, circiter 1 cm longis, saepe fissis, interioribus (3) oblongo-oblanceolatis ad ellipticis, obtusis, integris, pubescentibus, circiter 7 mm longis, 2 ad 4 mm latis; floribus 3, alabastro dense pubescenti, obovoideo; fructibus obovoideis ad subpyriformibus, haud stipitatis, glaberrimis, circiter 2.7 cm longis, 1.8 cm diametro, mesocarpo fibroso, endocarpo crustaceo, seminibus solitariis.

BRITISH NORTH BORNEO, Linkongan River, *No. 1216 D. D. Wood, col. P. Orolfo*, February 4, 1924 (type), in secondary forests, altitude about 60 meters; Kalumpang, *No. 1281 D. D. Wood, col. Puasa*, February 8, 1924.

A strongly marked species in the group with *Grewia omphacarpa* Miq. and *G. erythrocarpa* Ridl., characterized by its indumentum, consisting of dense short hairs and intermingled long spreading ciliate ones; its large, slenderly caudate-acuminate leaves which are abruptly rounded and cordate at the base; and its obovoid, glabrous, apparently fleshy, 1-seeded fruits. Named in honor of ex-Governor A. C. Pearson, C. M. G., of British North Borneo.

STERCULIACEÆ

Genus **PTEROSPERMUM** Schreber

Pterospermum diversifolium Blume.

Pterospermum diversifolium BLUME, Bijdr. (1825) 88.

BRUNEI, *D. D. Wood 1867, col. Goklin*, May, 1924. Indo-China and the Malay Peninsula through Malaysia to the Philippines and the Moluccas.

The form recorded from Borneo, on the authority of Miquel, as *Pterospermum acerifolium* Willd., an Indian species, is probably referable here, as Willdenow's species apparently does not extend to the Malay Archipelago.

GUTTIFERÆ

Genus **KAYEA** Wallich

Kayea acuminatissima sp. nov.

Arbor glaberrima, ramis teretibus, pallidis, circiter 3 mm diametro; foliis chartaceis vel subcoriaceis, oblongis, 14 ad 17 cm longis, circiter 5 cm latis, basi rotundatis ad subacutis, apice perspicue acutissime acuminatis, acuminis 1 ad 1.5 cm longis, apiculatis, in siccitate subolivaceis, utrinque nitidis, concoloribus, dense sed haud profunde subfoveolatis, nervis primariis utrinque circiter 15, tenuibus, curvatis, obscure arcuato-anastomosantibus;

petiolo circiter 1 cm longo; inflorescentiis axillaribus terminalibusque, 2 ad 6 cm longis, minoribus racemosis, majoribus paniculatis, ramis primariis brevibus, 3-floris; floribus albis, breviter (4 mm) pedicellatis, bibracteolatis, bracteolis oblongo-lanceolatis, acuminatis, circiter 1 mm longis; sepalis 4, exterioribus concavis, orbiculari-ovatis, circiter 4.5 mm longis, crassis, interioribus tenuioribus, laevis, orbiculari-obovatis; petalis 4, oblongo-ellipticis, 6 mm longis (immaturis); staminibus ∞, filamentis filiformibus, 5 ad 6 mm longis; stylo 6 mm longo. Fructibus ignotis.

BRUNEI, Tunggulian River, *D. D. Wood 1831, col. Goklin*, August 4, 1924, in swamps; locally known as *ladit*.

A species of the section *Eukayea*, well characterized by its very sharply acuminate leaves, the acumen being tipped by an almost spinelike apiculus.

MYRSINACEÆ

Genus ARDISIA Swartz

Ardisia subamplexicaulis sp. nov. § *Acrardisia*.

Frutex circiter 3 m altus, inflorescentiis minute puberulis exceptis glaber; ramulis teretibus, leviter lenticellatis, circiter 2 mm diametro; foliis alternis, chartaceis vel membranaceis, anguste oblongo-obovatis ad late oblanceolatis, integerrimis, sessilibus vel brevissime petiolatis, in siccitate olivaceo-viridibus, nitidis, utrinque concoloribus vel subtus paullo pallidioribus, 12 ad 22 cm longis, 5 ad 7 cm latis, apice acutis vel brevissime acuminatis, deorsum angustatis, basi abrupte rotundato-cordatis, 1 ad 2 cm latis, subamplexicaulibus, utrinque perspicue maculari-glandulosis; nervis primariis utrinque 15 ad 18, tenuibus, distinctis; inflorescentiis terminalibus, depauperato-paniculatis, circiter 10 cm longis, minute puberulis, ramis primariis paucis, 1 ad 1.5 cm longis, floribus subumbellatim dispositis; pedicellis 5 ad 7 mm longis, sursum leviter incrassatis; calycis circiter 4 mm diametro, lobis ovatis, acutis, glandulis paucis magnis instructis, circiter 1.5 mm longis, haud imbricatis, margine obscure ciliatis; corollae lobis oblongo-ovatis, subacutis, 3 mm longis, glandulis aurantiacis paucis magnis instructis; antheris ovato-lanceolatis, tenuiter acuminatis, 2.5 mm longis, connectivo perspicue atro-glanduloso; ovarium glabrum, atro-punctatum; stylis 2 mm longis, haud exsertis.

BRITISH NORTH BORNEO, Kalumpang, *D. D. Wood 1283, col. Puasa*, January 8, 1924, apparently from forests.

REVISION OF THE PHILIPPINE SPECIES OF THE GLENEINI (COLEOPTERA, LONGICORNIA)

By CHR. AURIVILLIUS

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The following paper on the Gleneini of the Philippine Islands is based on very rich material sent by C. F. Baker, Los Baños, Luzon, and on the collection of the Natural History Museum (Riksmuseum) in Stockholm.

I am very greatly obliged to Mr. Baker and to Prof. Y. Sjöstedt, the keeper of the Entomological Department of the Riksmuseum in Stockholm, for their liberality and courtesy.

Tribe GLENEINI Lacordaire

This tribe of the Lamiinæ is very nearly allied to the Saperdini and differs only by having the middle tibiæ furnished with a distinct furrow or incision on the outer side below the middle.

A rare species from the Philippine Islands (Luzon), described in the year 1841 by Westwood as *Colobothea leucospilota* and hitherto referred to the genus *Glenea*, belongs in fact to a new genus of the Saperdini, and thus is excluded from the Gleneini. I have thought it appropriate, however, to describe that genus in this paper.

Four genera of the Gleneini are known from the Philippine Islands.

Key to the Philippine genera of Gleneini.

a^1. Elytra abruptly deflexed at the sides; upper surface separated from the deflexed side by one or two keels ending in the outer apical spine.

b^1. Posterior tibiæ rounded..... Glenea Newman.

b^2. Posterior tibiæ compressed..... Chlorisanis Pascoe.

a^2. Elytra rounded at the sides, without keels or with the keels not reaching the outer apical angle.

b^1. Anterior claw of all the tarsi thickened and bifid, posterior claw simple. Heteroglenea Gahan.

b^2. All the claws simple..... Daphisia Pascoe.

Genus GLENEA Newman

The genus *Glenea* is very rich in species, occurring from western Africa through southern Asia to China and Japan and all the

islands as far as New Guinea, New Britain, New Caledonia, and North Australia.

Not less than 51 (54) species are known from the Philippine Islands and nearly all are endemic.

The species have been distributed in four subgenera,[1] of which three are represented in the Philippine fauna.

Key to the subgenera of Glenea.

a^1. Prothorax widening toward the base with the sides entirely straight. Eyes not tumid, never more protruding than the temples. Subg. **Macroglenea** Aurivillius.

a^2. Prothorax tapering toward the base or at the most cylindrical, always more or less constricted at sides near the base. Eyes tumid.

b^1. Scape without carina..... Subg. **Glenea** sens. str.

b^2. Scape on anterior side with a distinct carina. Subg. **Stiroglenea** Aurivillius.

Subgenus Macroglenea Aurivillius

The head, seen from above, has a peculiar form, being broadest at base and more or less tapering forward. Tarsi short; first joint of hind tarsi only as long as or shorter than the second and third together.

Key to the species of Glenea Newman (subgenus Macroglenea Aurivillius).

a^1. Scutellum triangular. Elytra chalybeate with white-tomentose spots. **G. beatrix** Thomson.

a^2. Scutellum transverse, very broadly rounded at apex. Elytra with yellow stripes or yellowish tomentum.

b^1. Prothorax with five yellow stripes. Elytra each with three yellowish stripes and a common sutural stripe; the discal stripe more or less abbreviated ..... **G. kraatzi** Thomson.

b^2. Prothorax above entirely clothed with a pale yellow tomentum and marked with two black dots. Elytra also yellow with some black dots and black humeral keel..... **G. sexpunctata sp. nov.**

Glenea beatrix Thomson.

Glenea beatrix THOMSON, Revue Zool. (3) 7 (1879) 4; RITS., Notes Leyden Mus. 3 (1893) 15; KUNTZEN, Ent. Rundschau 8 (1914) 31.

LUZON. MINDORO. BOHOL. MINDANAO.

Glenea kraatzi Thomson.

Glenea kraatzi THOMSON, Syst. Ceramb. (1865) 562.

Male, last ventral segment with a distinct keel near apex.

I wrongly identified this species with *G. regularis* in the Catalogue of the Lamiinæ. They are undoubtedly quite distinct,

[1] Aurivillius, Arkiv f. Zool. 13 (1920) 30–31.

and the latter species does not belong to the subgenus *Macroglenea*.

LUZON. "MINDANAO."

Glenea kraatzi Thomson var. **abbreviata** var. nov.

Differt vitta discali elytrorum brevissima, saepe triangulari.
PANAY. SIBUYAN.

Glenea (Macroglenea) sexpunctata sp. nov.

♀. Oculi supra approximati, vix tumidi; lobi inferiores genis haud duplo longiores. Tubercula antennigera approximata, sulco angulari separata. Caput pronoto angustius. Pronotum basin versus sensim latius, transversum, lateribus omnino rectis conicum. Scutellum late rotundatum. Elytra apice emarginata bispinosa costa humerali usque ad apicem distincta, infrahumerali obsoleta. Pedes mediocres; tarsi breves; articulus primus tarsorum posticoram 2° et 3° simul sumtis brevior. Unguiculi simplices. Virescente-nigra, tomento denso supra sulphureo, infra flavescente-griseo vestita; pronotum punctis duobus elytra punctis 4, duobus majoribus discalibus ante medium, duobus minoribus ad latera approximata pone medium, ornata. Prothorax utrinque vittis duabus, metasternum vitta laterali et abdomen utrinque maculis 4 denudatis aeneo-nigris praedita. Pedes cinereo-pubescentes. Antennae nigrae. Long. corporis 16 mm.

MINDANAO, Surigao, Surigao (*Baker*). Riksmuseum in Stockholm and Baker collection.

Very distinct in its coloration from all other species of the subgenus.

Subgenus **Glenea** Newman sensu str.

This subgenus comprises the majority of the species. The structural differences between the species are as a rule very slight, and I have therefore been compelled to found the smaller divisions mostly on markings and color. .

The most important structural differences are the breadth of the front, the length of the cheeks, the relative length of the scape and the third joint of the antennæ, the length of the hind tarsi, and the development of the lateral keels of the elytra. The keels are as a rule two, joined to each other a little before the apex and running to the outer apical spine. The upper or humeral keel is always distinct at base, but obtuse or obsolete

near apex, more seldom very distinct and acute in its entire length. The inferior or subhumeral keel is wanting or obsolete at base but thence more or less distinct, seldom very obsolete or entirely wanting, in which case the humeral keel is very acute and distinct from base to apex.

The pygidium or last dorsal segment of the male is normally entire and rounded, but in a few cases excised at apex and the last ventral segment at the same time with a large lobe on each side.

Key to the species of Glenea Newman sensu str.

a^1. Derm brilliant blue, chalybeate, greenish or violaceous, ornamented with white spots. Hind tarsi elongate with the first joint longer than the second and third together. Front somewhat higher than broad. Femora rufous.

b^1. Tibiæ and tarsi black. Antennæ fuscous. Humeral keel of the elytra united near apex to the subhumeral keel. Last abdominal segment metallic blue..... G. aphrodite Thomson.

b^2. Tibiæ and tarsi testaceous. Antennæ brownish. Humeral keel of the elytra ending free near apex. Last abdominal segment rufous. Male, claws appendiculate. Female, claws simple.

G. lepida Newman.

a^2. Derm not metallic.

b^1. Prothorax and elytra with blue, greenish, or metallic markings, or at least the sides of the metasternum with metallic scales.[3] Hind tarsi long and slender, bluish white above; first joint longer than the two following together and four to six times as long as broad at apex. Scutellum blue or greenish. Elytra with apical blue or grayish spot.

c^1. Elytra without bluish spots, only with narrow sutural and humeral stripes. Femora testaceous..... G. gracilis Aurivillius.

c^2. Elytra each with one to six (isolated) blue or greenish spots.

d^1. Elytra with only one elongate, oblique discal spot near base. Sutural stripe abbreviated at base, humeral stripe abbreviated posteriorly and more or less thickened at end. Legs black. Pronotum with three bluish stripes. Female, third antennal joint silvery blue..... G. artemis Aurivillius.

d^2. Elytra each with two to six isolated bluish or greenish (rounded) spots.

e^1. Sutural stripe continuous from base to apex. The markings of the elytra grayish or only slightly bluish. Prothorax with three bluish stripes. Humeral keel of the elytra acute to apex, subhumeral keel behind middle wanting or very obsolete.

[3] Cf. also *G. tritoleuca* Aurivillius in which the front and the stripes of the prothorax sometimes are slightly greenish.

f^1. Elytra with abbreviated humeral stripe, a short discal stripe at base and two isolated spots, the first discal before middle, the second lateral behind middle. Male, femora testaceous. **G. pagana sp. nov.**

f^2. Elytra each with two spots near base, two at middle and one lateral behind middle. Humeral stripe wanting, represented by the three lateral spots. Legs blackish, femora brownish at base....., **G. sordida Aurivillius.**

e^2. Sutural stripe wanting or only distinct behind the middle. Markings of the elytra blue, greenish, or margaritaceous.

f^1. Humeral stripe long and linear, ending somewhat behind middle and followed by a lateral spot.

g^1. Elytra with a linear discal stripe abbreviated at base and ending near middle; a small lateral spot at end of the humeral stripe ..... **G. magica Thomson.**

g^2. Elytra without discal stripe, instead of with two spots, one before, one near the middle.

G. benguetana sp. nov.

? G. lineella Thomson.

f^2. Humeral stripe wanting, represented by two or three large spots.

g^1. Lateral stripes of prothorax straight and horizontal; dorsal stripe continuous; basal margin not blue between the stripes. Legs testaceous..... **G. exculta Newman.**

g^2. Lateral stripes of prothorax oblique, much lower at anterior end; dorsal stripe often interrupted in the middle; basal margin as a rule with blue or metallic girdle. Tibiæ and tarsi black with pale blue pubescence; femora reddish or black..... **G. suavis Newman.**

b^2. Prothorax and elytra with white, gray, yellowish, brown, or black markings.

c^1. Elytra with the subhumeral keel distinct and near apex united to the humeral keel, which is more or less obtuse near apex.

d^1. Antennal joints (8) 9 to 11 white or pale yellowish. Elytra without spots.

e^1. Elytra only with sutural and humeral stripes. Prothorax on each side only with a single free white stripe.

G. astarte Thomson.

e^2. Elytra also with a narrow and somewhat obsolete discal stripe. Prothorax on each side with two narrow grayish stripes.

G. quinquevittata sp. nov.

d^2. Antennæ not whitish at apex.

e^1. Hind tarsi long and narrow; basal joint longer than the two following joints together and four to six times as long as broad at apex.

f^1. Elytra with yellow or white spots without stripes. Legs testaceous. Antennæ brown or fuscous.

g^1. Elytra black. Prothorax and vertex with a single yellow stripe.

h^1. Elytra each with three yellow spots, one near base, one near middle, and the third apical.
G. concinna Newman.

h^2. Elytra each with two yellow spots, one near middle, the other apical ..... **G. colenda** Thomson.

g^2. Elytra brown with the apical third black with two white spots. Vertex with two white stripes. Prothorax above with medial line and lateral series of three white dots.

f^2. Elytra at least with sutural stripe..... **G. lusoria** Pascoe.

g^1. Elytra with very broad orange yellow sutural stripe. Prothorax entirely clothed above with orange yellow tomentum without stripes. Legs black.
G. bangueyensis var. nigripes var. nov.

g^2. Elytra with sutural and humeral gray or yellow stripes; humeral stripe rarely dissolved in spots or obsolete.

h^1. Prothorax gray with black mesial stripe.
G. dido sp. nov.

h^2. Prothorax black with three pale stripes.

i^2. Elytra with a long and free discal stripe from base to beyond middle and free apical spot; stripes grayish ..... **G. iligana sp. nov.**

i^2. Elytra without discal stripe or only with a short discal stripe at base.

j^1. Vertex with a single broad yellow stripe. Mesial stripe of prothorax broad and yellow. Elytra without free spots.

k^1. Sutural stripe strongly widened behind the scutellum to a squarish spot, thence constricted and narrow. Humeral stripe linear, abbreviated posteriorly. Femora reddish.
G. minerva Aurivillius.

k^2. Sutural stripe of uniform breadth throughout or gradually broader toward the base. Humeral stripe rather broad and nearly reaching the apex. Legs black .... **G. univittata** Aurivillius.

j^2. Vertex with two pale narrow stripes or without stripes. Elytra often with free spots or with a short transverse fascia at middle.

k^1. Elytra at base with abbreviated discal stripe; their markings grayish. Femora reddish. Male, pygidium excised at apex; last ventral segment cleft into large lobes. Female, third antennal joint entirely blackish.
G. fissicauda sp. nov.
G. lobata sp. nov.

k^2. Elytra without discal stripe; their markings white or yellowish. Sutural and humeral stripes very narrow, linear or obsolete; the latter sometimes dissolved in two or three spots or lines; no other markings or a transverse line at middle.

Male, last abdominal segment normal. Female, third antennal joint with a bluish white ring at apex ..... G. tritoleuca Aurivillius.

e^2. Hind tarsi short and broad; basal joint triangular, shorter, or not longer than the two following joints together, at the most three times as long as broad at the apex.

f^1. Prothorax above nearly entirely yellow or orange.

g^1. Head and prothorax with fine black mesial line. Elytra above densely clothed with a yellow tomentum, shoulders and deflexed sides nearly glabrous and blackish. G. humeralis sp. nov.

g^2. Prothorax without mesial line; apical and basal margin narrowly black. Elytra black, each with three white stripes..... G. flavicollis sp. nov.

f^2. Prothorax striped above or entirely black.

g^1. Prothorax above at each side with a very broad white stripe and with a black mesial stripe, which is sometimes divided by a fine (interrupted) white line. Elytra black with sutural, humeral, and short discal stripes.

h^1. Stripes of the vertex parallel. Discal stripes of the elytra narrow and free.. G. cylindrepomoides Thomson.

h^2. Stripes of the vertex strongly divergent. Discal stripes of the elytra very broad and united to the base of the sutural stripe..... G. triangulifera sp. nov.

g^2. Prothorax above green or greenish with three pale stripes, the mesial sometimes obsolete..... G. viridis sp. nov.

g^3. Prothorax above black or brown with pale stripes or entirely black.

h^1. Elytra red-brown (at least before middle) with free white or yellowish spots or dots; humeral stripes wanting. Legs brown.

i^1. Apical part of elytra black with a transverse white spot behind middle and a large squarish white spot near apex; no stripes. Vertex with broad contiguous stripes. Mesial stripe of prothorax broad and white; sides white without free stripe. G. caraga Heller.

i^2. Elytra red-brown to apex.

j^1. Elytra without sutural stripe and apical spot. G. samarensis sp. nov.

j^2. Elytra with yellowish sutural stripe and apical spot. G. referens sp. nov.

h^2. Elytra black, blackish, or fuscous, rarely brown or yellowish in basal third, but in that case without spots or with only a transverse streak at middle.

i^1. Upper[1] lateral pale stripe of the prothorax, if present, nearly quite lateral, at its posterior end placed below the shoulder of the elytra and never in continua-

[1] If there are two lateral stripes.

tion with the discal stripe. Legs black. Antennæ entirely black.

j^2. Vertex with broad contiguous stripes. Prothorax with distinct stripes; the mesial stripe broad. Elytra with rather broad sutural and linear humeral stripes; discal stripes usually wanting. All the markings yellowish gray.

G. intermixta sp. nov.

j^2. Vertex with two narrow separate stripes or without stripes. Elytra without discal stripes.

k^1. Prothorax with three yellow stripes. Elytra with very distinct yellow or yellowish sutural and humeral stripes..... G. commixta Aurivillius.

k^2. Prothorax above and elytra black without markings or only with very fine and obsolete stripes.

G. maura Pascoe.

i^2. Upper lateral pale stripe of the prothorax nearly dorsal and posteriorly connected with the discal stripe of the elytra or reaching the middle of the elytra between scutellum and shoulders.

j^1. Femora reddish. Eyes very tumid. Elytra blackish without markings..... G. niveopectus sp. nov.

j^2. Legs black. Eyes slightly tumid. Prothorax and elytra with distinct pale stripes.

k^1. Discal stripes of the elytra long, at least reaching the middle or continued by a series of spots.

l^1. Discal stripes of the elytra straight, not curved or approaching the suture. Sutural stripe entire. Elytra without apical spot, only lined with white or yellow.

m^1. Discal stripes of the elytra thick and short, not reaching to the middle, but followed by a series of spots. All the markings of the upper side sulphur yellow.

G. flavotincta var. vel sp. nov.

m^2. Discal stripes of the elytra fine and linear, reaching to the middle or nearly to the apex. Markings gray or whitish.

G. albolineata Thomson.

l^2. Discal stripes of the elytra curved or oblique, approaching the suture. Elytra with apical spot and entire humeral stripe.

m^1. Elytra with distinct sutural stripe, which however does not reach the base. Discal stripes curved at base and before middle united to the sutural stripe, thence again free and reaching the apical spot. Markings gray or whitish.

G. curvilinea sp. nov.

m^2. Elytra without sutural stripe. Discal stripes oblique at base and nearly reaching the suture at middle, thence running close to the suture to apex. Markings of the upper side yellow..... **G. regularis Newman.**

k^2. Elytra without discal stripes or with a very short oblique stripe at base. Female, apex of the third antennal joint white.

l^1. Basal fourth of the elytra entirely clothed with a brown or grayish brown tomentum without stripes or spots. Sutural and humeral stripes distinct behind that patch. Stripes of vertex and prothorax brownish.

G. palauensis Aurivillius.

l^2. Basal part of the elytra not clothed with a brownish tomentum.

m^1. Vertex black without pale stripes. Elytra with the derm reddish brown at base; humeral and sutural stripes very narrow or wanting; no other markings. Prothorax with three narrow whitish stripes.

G. basalis Aurivillius.

m^2. Vertex with pale stripes. Prothorax with distinct stripes. Elytra with the sutural and humeral stripes long and a short oblique discal stripe at base.

G. versuta Newman.

c^2. Elytra with the subhumeral keel entirely wanting, at least behind the middle, and the humeral keel very distinct and acute to the apical spine, rarely obtuse at apex.

d^1. Elytra with the subhumeral keel entirely absent and the humeral keel rather obtuse near the somewhat declivous apex. Body with sulphur yellow markings. Elytra without stripes. Hind tarsi very short..... **G. pulchella Pascoe.**

d^2. Subhumeral keel of the elytra wanting only behind the middle; humeral keel very acute at apex. Markings gray or white.

e^1. Prothorax black above with three whitish stripes, the mesial broad. Vertex with two contiguous pale stripes. Elytra black, above with a broad discal stripe from base to apex, but without sutural and humeral stripes. Hind tarsi very long; first joint longer than the two following together and five to six times as long as broad at apex.

G. bivittata Aurivillius.

e^2. Prothorax cinereous with two oblong black dorsal stripes or spots, reaching neither the apical nor the basal margin.

f^1. Elytra not black at apex and without white transverse band behind the middle, cinereous with four large black spots. Sides of prothorax cinereous without black spot. Legs brownish. Hind tarsi long..... **G. cinerea Thomson.**

f'. Apical fifth of the elytra black, anteriorly limited by a transverse white band. Elytra from base to behind middle with humeral, discal (and sutural) grayish stripes. Sides of prothorax with a black dot. Legs black. Hind tarsi rather short..... G. colobotheoides Thomson.

Glenea aphrodite Thomson.

Glenea aphrodite THOMSON, Syst. Ceramb. (1865) 561.

LUZON, Laguna, Mount Banahao. MINDANAO.

Glenea lepida Newman.

Glenea lepida NEWMAN, Entomologist 1 (1842) 301.

LUZON. MINDANAO. BASILAN.

Glenea gracilis Aurivillius.

Glenea gracilis AURIVILLIUS, Arkiv f. Zool. 15 (1923) 37.

LUZON. MINDORO. LEYTE. SIARGAO. BUCAS. MINDANAO.

Glenea artemis Aurivillius.

Glenea artemis AURIVILLIUS, Arkiv f. Zool. 15 (1923) 37.

LUZON.

Glenea pagana sp. nov.

♂. Angusta, gracilis, fusca infra brunnea, infra omnino virescente-argenteo squamosa obsque maculis nigris, supra vittis maculisque griseis aut virescente-griseis ornata. Caput breve cum oculis tumidis pronoto latius, punctatum vittis duabus verticis viridibus ornatum; frons, genae et tempora tota virescentia. Oculorum lobi inferiores magni genis triplo longiores. Antennae ad basin late distantes, fuscae scapo plus minus rufescente. Prothorax subcylindricus prope basin leviter constrictus, punctatus, supra subnudus niger vittis tribus virescentibus, externis lateralibus. Scutellum obtusum vel quadratum, totum viride-squamosum. Elytra sublinearia, apice emarginato truncata, intus dentata, extus spinosa, costa humerali acuta, infra humerali obsoleta, praedita, vitta angusta continua suturali, vitta abbreviata discali ad basin, vitta brevi humerali nec basin nec partem tertiam apicalem attingente, macula discali ante medium, macula laterali pone medium maculaque apicali griseis ornata. Femora rufescentia; tibiae nigricantes; tarsi supra argenteo-grisei, articulos basalis posticoram 2° et 3° simul sumtis multo longior. Long. corporis 9 mm.

LUZON, Benguet, Baguio (*Baker*). Baker collection; Riksmuseum, Stockholm.

Perhaps the male of *Glenea benguetana* sp. nov.

Glenea sordida Aurivillius.

Glenea sordida AURIVILLIUS, Arkiv f. Zool. 15 (1923) 36.

LUZON, Nueva Vizcaya, Imugan.

Glenea magica Thomson.

Glenea magica THOMSON, Syst. Ceramb. (1865) 563.

MINDANAO. LUZON, "Manilla" (Thorey).

Glenea benguetana sp. nov.

♀. Nigra, femoribus rufis signatoris argenteo-viridibus ornata. Caput punctatum, breve, cum oculis pronoto latius, nigrum genis, temporibus infra, vitta utrinque frontis vittisque duabus postice approximatis verticis viridibus. Lobi inferiores oculorum genis plus duplo longiores. Antennae ad basin late distantes, nigrae articulis 5–11 albido-sericeis. Prothorax leviter transversus, prope basin constrictus, punctatus, fere nudus, niger vittis tribus angustis pectoreque albido-viridibus; vittae externae fere laterales. Scutellum obtusum omnino albido-viride. Elytra versus apicem modice angustata, apice emarginato-truncata et extus spinosa, costa infra humerali obsolete et postice fere deleta, a basi parte quinta apicali excepta rude punctata, in medio vitta humerali nec basin nec apicem attingente, pone medium vitta suturali singuloque maculis 4 viridibus (prima discali ante medium, secunda suturali prope medium, tertia pone medium laterali, quarta apicali) ornata. Pectus et abdomen viridi-squamosa, maculis magnis lateralibus denudatis nitidis nigris ornata. Tibiae et tarsi obscura argenteo-pubescentia; articulus basalis tarsorum posticorum 2° et 3° simul sumtis longior. Long. corporis 9–10 mm.

LUZON, Benguet, Baguio (*Baker*). Baker collection, and Riksmuseum in Stockholm.

Glenea lineella Thomson.

Glenea lineella THOMSON, Syst. Ceramb. (1865) 563.

MINDANAO. Unknown to me.

Glenea exculta Newman.

Glenea exculta NEWMAN, Entomologist 1 (1842) 302.
Glenea ? coryphaea THOMSON, Syst. Ceramb. (1865) 563.

LUZON, Laguna, Mount Banahao and Mount Maquiling.

Glenea suavis Newman.

Glenea suavis NEWMAN, Entomologist 1 (1842) 302.
Glenea decemguttata AURIVILLIUS, Arkiv f. Zool. 13 (1920) 33.

LUZON. SAMAR. SIARGAO. MINDANAO.

Glenea astarte Thomson.

Glenea astarte THOMSON, Syst. Ceramb. (1865) 562.

LUZON. NEGROS. MINDANAO. BASILAN.

Glenea lycoris Thomson.

Glenea lycoris THOMSON, Syst. Ceramb. (1865) 563.

MINDANAO. Not seen by me. = *G. astarte* ♀ ?

Glenea quinquevittata sp. nov.

Fusca elytris brunneis, supra pallide vittata, infra albo- vel cinereo-tomentosa; antennae nigrae articulis 9–11 albis; femora testacea, tibiae tarsique nigricantia. Caput breve, cum oculis tumidis pronoto latius. Frons subquadrata (♂) vel quadrata (♀), griseo-pubescens utrinque flavido-vittata. Genae albido-tomentosae lobis inferioribus oculorum vix (♀) vel multo breviores. Vertex vittis duabus parallelis ochraceis ornatus. Prothorax prope basin leviter constrictus, basin versus vix (♂) vel leviter angustatus (♀) vitta media lata ochracea et utrinque vittis binis angustis bene separatis griseis ornatus. Scutellum rotundatum ochraceum. Elytra punctata punctis apicem versus sensim evanescentibus, apice oblique emarginato-truncata angulo externo spinoso costis lateralibus apicem versus valde obtusis et obsoletis, brunnea vitta suturali vittisque utrinque binis (vitta discali tenui subundulata, postice obsoleta, vitta humerali latiore apicem fere attingente) griseis. Tarsi postici breves articulo primo 2° et 3° simul sumtis brevior. Long. corporis 10–12 mm.

MINDANAO, Butuan (*Baker*). Riksmuseum in Stockholm and Baker collection.

Nearly allied to *G. astarte* Thomson but differing in having a discal stripe on the elytra, two lateral grayish stripes on the prothorax and the dorsal stripe on the prothorax ochraceous.

Glenea concinna Newman.

Glenea concinna NEWMAN, Entomologist 1 (1842) 301.
Glenea severa THOMSON, Syst. Ceramb. (1865) 565.

LUZON.

Glenea colenda Thomson.

Glenea colenda THOMSON, Rev. Zool. (3) 7 (1879) 18.

MINDANAO. LUZON, "Manilla" (Thorey).

Glenea lusoria Pascoe.

Glenea lusoria PASCOE, Trans. Ent. Soc. London (3) 3 (1867) 405, nota.
Glenea bimaculata AURIVILLIUS, Arkiv f. Zool. 13 (1920) 34.

Philippines.

Glenea bangueyensis Aurivillius.

Glenea bangeyensis AURIVILLIUS, Arkiv f. Zool. 13 (1920) 35.

BORNEO. BANGUEY ISLAND.

Glenea bangueyensis Aurivillius var. nigripes var. nov.

A forma typica differt pedibus totis nigris genisque paullo longioribus lobis inferioribus oculorum triplo brevioribus. Long. corporis 12 mm.

NEGROS (*Baker*). Baker collection.

Glenea dido sp. nov.

♀. Nigro-fusca, infra albido-tomentosa, supra griseo-signata. Caput breve, cum oculis pronoto latius genis, temporibus, vitta utrinque laterali frontis vittisque duabus curvatis oculos cingentibus verticis griseis vel albidis. Prothorax latitudine basali fere longior pone medium leviter constrictus, dense punctulatus, griseo-pubescens vittaque media dorsali basin haud attingente nigra ornatus. Scutellum obtusum, nigrum macula apicali grisea. Elytra apicem versus angustata, apice emarginato-truncata, a basi usque ad quintam partem apicalem rude punctata costis lateralibus distinctis et ante apicem conjunctis, macula apicali vittisque binis latis (discali postice cum macula apicali conjuncta et humerali ante apicem desinente) ornata; sutura a basi ultra medium anguste nigra. Pedes flavescente-fusci; tarsi mediocres, articulus basalis posticorum 2° et 3° simul sumtis vix longior. Antennae latae fuscae. Long. corporis 11 mm.

MINDANAO, Port Banga. Riksmuseum in Stockholm.

Glenea iligana sp. nov.

♀. Nigra, supra albido-signata, infra dense cinereo-pubescens. Caput breve, punctatum, nigrum, genis, temporibus, vitta utrinque frontis vittisque duabus approximatis, parallelis, rectis verticis cinereis. Oculi tumidi; lobi inferiores genis vix sesqui longiores. Tubercula antennifera late distantia, parum producta. Scapus antennarum articulo 3° multo brevior. Prothorax punctatus, subquadratus, ante basin modice constrictus, capite vix angustior, vittis tribus angustis dorsalibus, plus minus interruptis punctoque laterali cinereo-albidis ornatus. Scutellum obtusum albido-pubescens. Elytra apicem versus modice angustata, apice recte truncata, bispinosa spina exteriore longiore, vitta angusta suturali, vittis ternis nec basin nec apicem attingentibus (prima discali, secunda humerali, tertia subhumerali) maculaque apicali albido-cinereis ornata; epipleura etiam ante medium albida; costae laterales distinctae, prope apicem obtusae et conjunctae. Tarsi postici elongati articulus basalis 2° et 3° simul sumtis multo longior. Abdomen utrinque maculis 4 obscuris fere denudatis praeditum. Long. corporis 13 mm.

MINDANAO, Lanao, Iligan (*Baker*). Baker collection.

Glenea minerva Aurivillius.

Glenea minerva AURIVILLIUS, Tijdschr. v. Ent. 65 (1922) 171.

PALAWAN.

Glenea univittata Aurivillius.

Glenea univittata AURIVILLIUS, Arkiv f. Zool. 15 (1923) 38, 40.

Glenea univittata ab. vinculata ab. nov.

Elytra ad basin vitta brevi discali instructa.

LUZON. MASBATE, Sorsogon, Aroroy. SIBUYAN. NEGROS.

Glenea fissicauda sp. nov.

Nigra, supra albido-vittata et maculata, infra griseo-pubescens aut albido-tomentosa; pedes rufi, tarsis fuscis. Frons griseo-pubescens utrinque albido-vittata, punctulata, in more latitudine altior, in femina lata, subquadrata. Genae moris lobis inferioribus triplo, feminae haud duplo breviores. Vertex albido-bivittatus, vittis parallelis. Prothorax subcylindricus, ante basin constrictus, leviter punctatus, albo trivittatus, vittis externis lateralibus, a supero vix aut salum ex porte discernendis. Scu-

tellum obtusum albido-tomentosum. Elytra apice recte truncata bispinosa spina suturali breviore costis lateralibus distinctis et prope apicem conjunctis, cinereo-pubescentia vitta suturali et utrinque vittis duabus albidis ornata, vitta discali saepissime abbreviata medium haud attingente, vitta humerali ante apicem desinente ibique plus minus incrassata, interdum interrupta linea media basin haud attingente et macula laterali indicata, macula apicali grisea. Tarsi breves, supra griseo-pubescentes; posticorum articulos primus duabus sequentibus simul sumtis haud vel vix longior. Vitta lateralis metasterni maculaque laterales abdominis fuscae. Long. corporis 11–12 mm.

♂. Pygidium apice profunde incisum. Segmentum ventrale ultimum infra fere ad basin fissum, lobis lateralibus maximis, latis, apice late oblique subtruncatis et longe pilosis. Paramera longe exserta, lanceolata, infra pilis longis vestita.

NEGROS, Cuernos Mountains (*Baker*). Baker collection; Riksmuseum in Stockholm.

This and the following species differ from all other forms in the formation of the last (visible) abdominal segment of the male. It is to be noted that similar very sharp sexual differences occur also in some species of the South American genus *Colobothea*.

Glenea lobata sp. nov.

Speciei praecedenti valde similis et affinis. Femina vix nisi elytris subnudis, fortius punctatis vittaque suturali latiore, mas pygidio apice minus exciso lobisque lateralibus segmenti ventralis ultimi apice late emarginatis et utrinque ad angulos productis differunt. Long. corporis 11–12 mm.

MINDANAO. SAMAR. NEGROS, 1 male (*Baker*). Baker collection; Riksmuseum in Stockholm.

Glenea lobata ab. (?) biguttulata ab. nov. (sp. ?).

Pronotum utrinque inter vittas dorsales guttis binis albis notatum.

BASILAN (*Baker*). Only a single male, Baker collection.

Glenea triotoleuca Aurivillius.

Glenea tritoleuca AURIVILLIUS, Arkiv f. Zool. 15 (1923) 38, 40.
Glenea tritoleuca Aurivillius var. *tripartita* AURIVILLIUS, Arkiv f. Zool. 15 (1923) 39.

MINDANAO. BASILAN.

Glenea humeralis sp. nov.

Nigro-fusca femoribus ad basin flavidis, supra ochraceo-, infra (flavescente) cinereo-tomentosa. Caput breve, cum oculis pronoto latius, ochraceo pubescens vitta frontis et verticis nigra genisque cinereis. Genae mediocres lobis oculorum parum breviores. Prothorax leviter transversus basin versus angustatus, ante basin leviter constrictus supra totus ochraceus linea tenue media basin haud attingente fusca ornata lateribus infra cinereis vitta nulla laterali. Scutellum obtusum ochraceum. Elytra apice recte truncata extus spinosa supra usque ad costam humeralem dense ochraceo-tomentosa, lateribus deflexis areaque subquadrata humerali denudatis nitidis brunneo-nigris, carinis lateralibus distinctis prope apicem conjunctis. Tarsi breves; posticorum articulus basalis 2° et 3° simul sumtis brevior. Long. corporis 11 mm.

POLILLO (*Baker*). Baker collection.

Resembles *G. sulla* Aurivillius from Borneo, but without the lateral black stripe on the prothorax and with the ochraceous humeral stripe of the elytra completely united to the discal area. Also the tarsi are shorter than in *G. sulla*.

Glenea flavicollis sp. nov.

♀. Nigra, supra albido-vittata pronoto fere toto aurantiaco, infra dense albo-pubescens vitta laterali, in ventre maculari denudata nigra ornata. Pedes cinereo-pubescentes. Antennarum articuli 1–3 nigri (reliqui desunt). Caput punctatum genis, temporibus vittaque utrinque frontis et verticis albidis; vittae verticis late separatae. Prothorax leviter transversus, supra, dense et laete aurantiaco-tomentosus margine apicali et basali anguste nigris plus minus albido pubescentibus, utrinque vitta infralaterali curvata nigra instructus. Scutellum late rotundatum, albido pubescens. Elytra a basi ultra medium rude punctata, deinde fere laevia vitta communi suturali vittisque binis bene definitis albidis ornata, vitta intermedia discali paullo pone medium desinente, vitta humerali in spinam exeunte et apice cum vitta suturali conjuncta; carinis lateralibus distinctis ante apicem conjunctis. Tarsi breves; articulus primus posticorum 2♀ et 3° simul sumtis brevior. Genae lobis inferioribus oculorum haud duplo breviores. Long. corporis 14 mm.

SIBUYAN (*Baker*). Baker collection.

Only a single female of this species is known to me. It differs from all other species of the genus in the color of the prothorax.

No punctures are visible on the upper side of the prothorax.

Glenea cylindrepomoides Thomson.

Glenea cylindrepomoides THOMSON, Syst. Ceramb. (1865) 564.

LUZON.

Glenea triangulifera sp. nov.

Nigra, supra flavo-albido-vittata, infra griseo- vel albido-tomentosa et nigro-maculata. Caput breve, cum oculis tumidis pronoto latius. Frons latitudine altior, utrinque late flavido-vittata, in medio nigro-vittata. Genae nudae nigrae (♀) aut tomentosae (♂). Tempora tomentosa. Vertex vittis duabus postice valde divergentibus flavidis, inter eas triangulariter niger. Prothorax albido-tomentosus vitta lata dorsali vittaque utrinque infra-laterali nigris. Elytra punctata, apice truncata et extus spinosa, carinis lateralibus distinctis ante apicem conjunctis, vitta lata suturali, vitta latissima et brevissima discali cum vitta suturali conjuncta et maculam basalem transversam formante vittaque humerali nec basin nec apicem attingente flavo-albidis ornata. Pedes fusci (♂) aut rufo-testacei (♀); tarsorum posticorum articulus basalis 2° et 3° simul sumtis haud longior. Long. corporis 9–12 mm.

MINDANAO, Bukidnon, Tangkulan. NEGROS, Cuernos Mountains (*Baker*). Baker collection.

Easily distinguished from all other Philippine species of the genus by the black dorsal stripe of the prothorax and the broad black triangle of the vertex. The markings of the elytra nearly agree with those of *Glenea minerva* from Palawan.

Glenea viridis sp. nov.

Viridis, opaca (haud metallica) supra albido-vittata, infra dense albo-tomentosa; antennae apicem versus nigricantes; pedes pube tenui cinerea vestiti, femora ima basi testacea. Frons et vertex bivittata. Genae et tempora albida. Prothorax 4-vittatus, vitta superiore latiore. Scutellum obtusum, viride. Elytra apice emarginata et extus spinosa utrinque bicarinata, carinis postice conjunctis et in spinam exeuntibus, vitta suturali (interdum obsoleta) vittisque ternis (discali abbreviata, humerali lata apicem versus dilatata, infrahumerali tenui in mare fere nulla) albidis ornata. Tarsi breves, posticorum articulas primus 2° et 3° simul sumtis brevior. Long. corporis 11–13 mm.

♂. Metasternum in medio foveis duabus contiguis dense fulvo-tomentosis impressum.

SAMAR. MINDANAO (*Baker*). Baker collection; Riksmuseum in Stockholm.

The only species known with a green body.

Glenea caraga Heller.

Glenea caraga HELLER, Philip. Journ. Sci. 19 (1921) 541, t. 2, f. 3.

MINDANAO. SAMAR.

Glenea samarensis sp. nov.

♀. Brunnea, supra vittis et maculis flavido-tomentosis, infra tomento flavescente vestita. Caput cum oculis pronoto vix latius. Frons quadrata griseo-pubescens, utrinque flavo-vittata; genae griseae, mediocres, lobis oculorum haud duplo breviores; tempora flavida; vertex vittis duabus parallelis flavidis. Prothorax subquadratus, prope basin constrictus, flavido-trivittatus vittis lateralibus latis, inter vittas discrete punctatus. Scutellum late rotundatum, totum flavidum. Elytra apicem versus modice angustata, apice late truncata, bispinosa spina externa elongata, carinis lateralibus distinctis postice obtusis, prope apicem conjunctis, maculis quaternis (prima discali prope basin elongata, secunda fere in medio ad suturam approximata, tertia pone medium prope carinam humeralem rotundata, quartaque parva laterali prope apicem) punctaque uno alterave flavidis ornata. Antennae fusco-brunneae. Pedes brunnei tenuiter cinereo-pubescentis; tarsi breves, posticorum articulus basalis 2° et 3° simul sumtis brevior. Abdomen maculis trigonis lateralibus albo-tomentosis, praeterea griseo-pubescens. Long. corporis 13 mm.

SAMAR (*Baker*). Baker collection.

A single female only.

Glenea referens sp. nov.

♂. Brunnea capite et pronoto nigricantibus, supra vittis maculis flavidis ornata, infra albido-tomentosa. Frons latitudine altior albido-tomentosa vitta media angusta fusca. Genae subnudae, lobis oculorum quadruplo breviores. Tempora flavida. Vertex vittis duabus bene separatis flavidis. Caput breve cum tumidis pronoto latius. Prothorax subcylindricus, latitudine basali longior lateribus leviter convexis, prope basin paullulum constrictus, vitta dorsali vittaque utrinque laterali latissima coxas fere attingente flavidis ornatis. Scutellum subtruncatum, totum flavidum. Elytra fere ad apicen rude, ad basin foveatim punctata, apice emarginato-truncata angulo suturali dentato, externo

spina brevi ornato, carinis lateralibus distinctis prope apicem conjunctis praedita, vitta suturali postice in maculam apicalem dilatata maculisque quaternis rotundatis (prima discali prope basin, secunda parva laterali ante medium, tertia fere in medio vittam suturalem tangente, quarta minore laterali pone medium) flavido-tomentosis ornata. Corpus infra in medio pallidius, abdomen maculis lateralibus brunneis instructum. Tarsi breves, posticorum articulus basalis 2° et 3° simul sumtis haud longior. Antennae apicem versus infuscatae. Long. corporis 11 mm.

MINDANAO, Lanao, Kolambugan (*Baker*). Baker collection.

Nearly allied to *G. samarensis*, but probably not the male of that species, the elytra being more strongly punctured and having a distinct sutural stripe, an apical spot, and differently arranged markings. A small yellowish stain at base between scutellum and the shoulders.

A female specimen from Luzon, which differs only by having the front broader, subquadrate with broad medial black stripe, longer brown cheeks, much smaller elytral spots, but these arranged exactly in a similar way, and the ordinary sexual markings, may be the true female of *G. referens*.

Glenea helleri Aurivillius.

Glenea helleri AURIVILLIUS, Cat. Col. 74 (1923) 506.
Glenea scalaris HELLER, Philip. Journ. Sci. 19 (1921) 541, t. 2, f. 4.

LUZON. Not seen by me.

Glenea intermixta sp. nov.

Nigro-fusca (elytris interdum brunneis, immatura?), supra albo-vittata, infra griseo- et albido-tomentosa femoribus ad basin rufis. Caput punctatum cum oculis tumidis pronoto latius. Frons latitudine altior, in medio subnuda, utrinque flavescente vittata. Genae tenue pubescentes, lobis inferioribus oculorum parum (♀) vel plus duplo breviores. Vertex vittis duabus omnino contiguis antice inter antennal divergentibus ornatus. Antennae totae fuscae vel fusco-brunneae. Prothorax subcylindricus vitta lata dorsali vittaque angusta laterali infra pube cinerea, determinata albis instructus. Scutellum obtuse rotundatum, dense albo-tomentosum. Elytra ante medium fortius punctata, apice truncata et extus spinosa, carina humerali usque ad spinam acuta et continua, carina subhumerali obtusa ante apicem omnino evanescente, vitta lata suturali saepissime in medio fasciola transversa connexa vittaque tenui humerali nec basin nec apicem attingente albidis ornata, inter-

dum etiam vitta brevi discali ad basin instructa. Pectus et abdomen maculis denudatis fuscis praedita. Tarsorum posticorum articulus basalis 2° et 3° simul sumtis haud (♀) vel parum (♂) longior. Long. corporis 9–12 mm.

MINDANAO, Zamboanga, Dapitan, Iligan. BASILAN (*Baker*). Riksmuseum in Stockholm and Baker collection.

Allied to *Glenea commixta* Aurivillius and *univittata* Aurivillius, but differing in having the humeral keel of the elytra sharp to the apex and the subhumeral keel disappearing before apex. The stripes of the upper side are white or whitish, rarely yellowish.

Glenea commixta Aurivillius.

Glenea commixta AURIVILLIUS, Arkiv f. Zool. 15 (1923) 37, 40.

MASBATE, Sorsogon, Aroroy, Masbate. SAMAR.

Glenea commixta ab. (var. ?) **fasciola** Aurivillius.

Glenea commixta ab. (var. ?) *fasciola* AURIVILLIUS, Arkiv f. Zool. 15 (1923) 38.

MINDANAO.

Glenea maura Pascoe.

Glenea maura PASCOE, Trans. Ent. Soc. London (3) 3 (1867) 405, nota.

MINDANAO.

Glenea niveopectus sp. nov.

♂. Nigro-fusca, supra fere unicolor infra dense niveo-tomentosa utrinque maculis 4 parvis lateralibus denudatis abdominis brunneis. Caput breve cum oculis tumidis pronoto multo latius. Frons quadrata, punctata, cana, utrinque albido-vittata. Genae et tempora albido-tomentosa; genae lobis oculorum duplo breviores. Antennae totae nigrofuscae; scapus articulo tertio haud brevior. Prothorax subquadratus, supra nigro-fuscus, punctulatus lineis tribus, externis obsoletis, pallidis ornatus, utrinque in lateribus omnino niveo-tomentosus absque vitta. Scutellum latum, obtusum, nigrum linea tenui media alba. Elytra tota nigro-fusca seriatim punctata, apice suboblique truncata angulo suturali vix dentato, externo spinoso, carinis lateralibus distinctis ante apicem conjunctis instructa, unicolora margine apicali tenuissime albido-ciliato. Femora coxae et dimidium basale tibiarum posticorum rufa; tibiae tarsique fusca; tarsi breves, articulus basalis posticorum 2° et 3° simul sumtis multo brevior. Long. corporis 8 mm.

BASILAN (*Baker*). Baker collection.

A single male. Resembling *Glenea maura* Pascoe, but at once distinguished by its much broader head, shorter prothorax, reddish femora, and the entirely white sides of the prothorax.

Glenea albolineata Thomson var. **mindanaonis** var. nov.

Violascente-nigra supra albovittata, infra dense albo-tomentosa maculis lateralibus denudatis nigris. Caput pronoto haud latius oculis haud vel vix tumidis. Frons latitudine altior, utrinque vitta albida; in medio punctata, nuda. Genae lobis inferioribus oculorum parum breviores nudae (♀) vel albo-pubescentes (♂). Tempora vitta obliqua albida. Vertex 4-vittatus; vittae intermediae tenues parallelae, laterales pone oculos breves latiusculae. Antennae ad basin modice distantes tuberculis distinctis, corpore longiores, nigrae. Scutellum macula media apicali albida. Elytra a basi usque ad medium punctata, pone medium fere laevia, apice subtruncata angulo externo spinoso, suturali dentato, carinis lateralibus postice obtusis subaequalibus, prope apicem conjunctis, vittis 7 rectis, bene separatis, linearibus, optime definitis ornata [vitta communi suturali, vitta discali medium (♀) vel apicem fere (♂) attingente, vitta humerali apice libera (♀) vel cum fascia apicali connexa (♂) vittaque infrahumerali inter carinas] margine apicali etiam albido. Pedes cinereo-pubescentes; tarsi breves, posticorum articulus basalis 2° et 3° simul sumtis brevior vel haud longior. Long. corporis 10–14 mm.

♂. Segmentum ultimum ventrale apice convexum vel obtuse carinatum.

MINDANAO, Surigao, Surigao: Agusan, Butuan. SAMAR (*Baker*). Riksmuseum, Stockholm; Baker collection.

Specimens from Bouru differ by having the white stripes of the prothorax much broader and the stripes of the elytra more or less united, at least at apex. In a male from Bouru the last ventral segment is very long, fornicate and distinctly carinate at apex.

Glenea flavotincta var. nov. vel sp. nov.

As a very doubtful form of *G. albolineata* I regard two females, one from Samar in Baker's collection and one from Mindanao in the Riksmuseum, Stockholm. They differ in having all the stripes of the upper side yellow, the discal and humeral stripes of the elytra much broader and posteriorly more or less dissolved in rounded or irregular spots. In the specimen from Samar the humeral stripe is continuous and the discal only at and behind

the middle represented by two or three dots, but in the specimen from Mindanao both stripes are, from the middle, replaced by very irregular yellow spots. The most important difference, however, is that the forehead is much broader and the cheeks are shorter than in *G. albolineata*.

Glenea curvilinea sp. nov.

♂. Nigra, supra griseo-vittata, infra omnino albido-tomentosa maculis nullis pedibus cinereo-pubescentibus. Caput cum oculis tumidiusculis pronoto vix latius. Frons latitudine parum altior. Genae mediocres, lobis inferioribus oculorum haud duplo breviores. Antennae ad basin distantes, corpore longiores nigrae. Caput totum albido-tomentosum vitta media verticis nigra. Prothorax subcylindricus, prope basin leviter constrictus, supra albido-trivittatus vittis externis latis. Scutellum albido-tomentosum. Elytra apice emarginato-truncata angulo externo spina ornato, costis lateralibus apice conjunctis parum distinctis, vittis ternis (prima lata ad basin discali, mox autem versus suturam curvata et eam usque ad apicem adjuncta, secunda humerali maculam apicalem attingente, tertia latera deflexa fere omnino occupante) maculaque apicale griseis ornata, ante medium punctata pone medium fere laevia. Segmentum ventrale ultimum infra planum. Long. corporis 10–11 mm.

MINDANAO, Agusan, Butuan (*Baker*). Riksmuseum in Stockholm and Baker collection.

Nearly allied to *G. albolineata* Thomson, but distinct by having the discal stripe of the elytra curved at the base and soon reaching the suture. I have not seen the female.

Glenea regularis Newman.

Glenea regularis NEWMAN, Entomologist 1 (1842) 302.

LUZON; Camarines Sur, Mount Isarog: "Manilla" (Thorey): Laguna, Mount Maquiling.

Glenea palauensis Aurivillius.

Glenea palauensis AURIVILLIUS, Arkiv f. Zool. 1 (1903) 325, fig. 29.

PALAWAN.

Glenea basalis Aurivillius.

Glenea basalis AURIVILLIUS, Arkiv f. Zool. 15 (1923) 39.

LUZON.

Glenea versuta Newman.

Glenea versuta NEWMAN, Entomologist 1 (1842) 302; Arkiv f. Zool. 15 (1923) 39, 40.

LUZON, Sorsogon, Aroroy. POLILLO.

Glenea versuta Newman ab. bipunctata Aurivillius.

Glenea versuta Newman, ab. *bipunctata* AURIVILLIUS, Arkiv f. Zool. 15 (1923) 39.

LUZON, Sorsogon, Aroroy. SAMAR.

Glenea versuta Newman ab. ♀ fasciolata Aurivillius.

Glenea versuta Newman ab. ♀ *fasciolata* AURIVILLIUS, Arkiv f. Zool. 15 (1923) 39.

MINDANAO. SIARGAO. BASILAN.

Glenea pulchella Pascoe.

Glenea pulchella PASCOE, Trans. Ent. Soc. London (2) 4 (1857) 260.
Glenea vesta PASCOE, Proc. Zool. Soc. London (1866) 260, t. 28, f. 3; Trans. Ent. Soc. London (3) 3 (1867) 411.

MINDANAO. MALACCA. BORNEO. MOLUCCAS.

Specimens from Mindanao have a small sulphur yellow lateral dot on the elytra behind the middle; this dot is wanting in specimens from Borneo and Malacca but still more developed in a specimen from Ceram.

Pascoe altered the name *pulchella* to *vesta* under the supposition that *G. pulchella* Hope was an older name; but Hope's species was not described before 1860, when it was introduced by Thomson as *G. pulchella*. Thomson's species may therefore be named *G. pulchra*.

Glenea bivittata Aurivillius.

Glenea bivittata AURIVILLIUS, Arkiv f. Zool. 1 (1904) 326, fig. 30.

PALAWAN.

Glenea cinerea Thomson.

Glenea cinerea THOMSON, Syst. Ceramb. (1865) 565.

LUZON. MINDORO.

A somewhat variable species. The four black spots of the elytra large and squarish or smaller and rounded.

Glenea colobotheoides Thomson.

Glenea colobotheoides THOMSON, Syst. Ceramb. (1865) 562.

LUZON. SIARGAO. MINDANAO. BASILAN.

The following three species are unknown to me:

Glenea glauca Newman.

Glenea glauca NEWMAN, Entomologist 1 (1842) 302.

LUZON, Manila.

Glenea stellata Thomson.

Glenea stellata THOMSON, Syst. Ceramb. (1865) 563.

"BORNEO?" MINDANAO?.

Glenea varifascia Thomson.

Glenea varifascia THOMSON, Syst. Ceramb. (1865) 562.

MINDANAO. ? = *regularis* Newm.

Glenea ana Thomson and *ochraceovittata* Thomson have been reported from the Philippine Islands, but were probably wrongly named.

Subgenus Stiroglenea Aurivillius

The majority of the species belonging to this subgenus have the same colors and markings as have the well-known *G. cantori* Fabricius from China and *G. angerona* Thomson from Java. Front broad and subquadrate. Hind tarsi short. Prothorax short, strongly constricted behind middle. Eyes moderately tumid. Humeral keel of the elytra ending free near apex; subhumeral keel distinct and acute at apex.

One species only is known from the Philippine Islands.

Glenea (Stiroglenea) luzonica sp. nov.

Nigra, albido pubescens; elytra (parte 5ª apicali nigra excepta) femora et tibiae anteriora abdomeneque rufo-brunnea. Caput albido-tomentosum macula frontis et vitta media verticis nigris. Prothorax albido-tomentosus, supra facia transversa basali maculisque duabus subquadratis ante medium, utrinque in latere guttis 4 denudatis nigris. Scutellum nigrum. Elytra leviter griseo pubescentia macula apicali cano-tomentosa. Latera pectoris nigro-guttata. Segmenta ventralia 1–4 utrinque late denudata. A *G. angerona* Thomson, cui proxime affinis, parte nigra apicali elytrorum multo breviore et signatoris prothoracis diversa. Long. corporis 11 mm.

LUZON, Camarines Sur, Mount Isarog. Riksmuseum, Stockholm, 1 female.

Genus **HETEROGLENEA** Gahan

Head broad with tumid eyes. Hind tarsi short.

Heteroglenea glechoma Pascoe.

Heteroglenea glechoma PASCOE, Trans. Ent. Soc. London (3) 3 (1867) 409; GAHAN, Ann. Nat. Hist. (6) 19 (1897) 490.

Heteroglenea fuscovirgata FAIRMAIRE, Ann. Soc. Ent. Belg. 27 (1883) 53.

MINDANAO. May easily be mistaken for a *Daphisia*.

Genus **DAPHISIA** Pascoe

The species are as a rule smaller than the species of *Glenea* and easily known by the rounded sides of the elytra. Head

broad with tumid eyes. Hind tarsi generally short. Lateral keels of the elytra wanting or obsolete, never reaching the apex.

Key to the species of Daphisia Pascoe.

a^1. Elytra truncate or slightly rounded at apex, unarmed. First joint of hind tarsi at least as long as the two following together. Elytra with a broad sutural stripe, a short discal stripe at the base sometimes united to the sutural stripe, a discal spot near middle, a humeral stripe not reaching the base and usually thickened at its posterior end and an apical spot gray or yellowish gray. All the markings sometimes united and nearly concealed by a grayish or yellowish tomentum (ab. *confluens*). Femora pale reddish at least at base..... D. discimaculata Aurivillius.

a^2. Elytra truncate with the exterior angle dentate or spined. Elytra without stripes. Scutellum white.

b^1. Larger, 9 to 10 millimeters. Entirely pale brown with two large white spots on each side of the breast..... D. brunnea sp. nov.

b^2. Smaller, 6 to 8 millimeters. Black; prothorax with three white stripes, elytra as a rule with two spots near base and a transverse fascia behind the middle white..... D. bakeri sp. nov.

Daphisia discimaculata Aurivillius.

Daphisia discimaculata Aurivillius, Arkiv f. Zool. 15 (1923) 41.
Daphisia discimaculata ab. ♀ *confluens* Aurivillius, Arkiv f. Zool. 15 (1923) 41.

Mindanao.

Daphisia brunnea sp. nov.

♀. Tota testaceo-brunnea, brunneo-pubescens, scutello maculisque utrinque 4 lateralibus pectoris et abdominis (prima in mesosterno, secunda in metasterno, tertia parva in segmento ventrali primo, quarta elongata in segmento ultimo) dense albo-tomentosis. Frons lata, subtransversa, punctata. Genae lobis inferioribus oculorum fere longiores. Antennae corpore parum longiores; scapus articulo 3° haud brevior. Pronotum subtransversum, basin versus levissime angustatus et ante basin obsolete constrictus, ante scutellum albo-tomentosum. Scutellum semiorbiculare. Elytra utrinque a basi ultra medium obtuse bicarinata carinis ante apicem evanescentibus, apicem versus parum angustata, apice truncata et extus spinosa, subseriatim punctata quarta parte apicali fere laevi. Tarsi breves. Unguiculi simplices. Long. corporis 9–10 mm.

Samar (*Baker*). Baker collection; Riksmuseum, Stockholm.

A very distinct species, forming a connecting link between *Glenea* and *Daphisia*.

Daphisia bakeri sp. nov.

Nigro-fusca, supra albo-vittata et -maculata, infra albido-pubescens vitta laterali pectoris et abdominis dense albo-tomentosa; pedes toti pallidi aut plus minus infuscati. Caput cum oculis pronoto latius. Frons subquadrata, punctulata, grisea; genae et tempora alba; vertex unicolor, fuscus. Oculi late distantes, mediocres; lobi inferiores genis duplo (♂) vel parum (♀) longiores. Antennae fuscae, corpore duple (♂) aut sesqui longiores; scapus articulo 3° parum brevior, interdum rufescens. Pronotum dense punctulatum, basin versus leviter angustatum lateribus rectis, albido-trivittatus vitta media tenui vel interrupta. Scutellum obtusum dense albo-tomentosum. Elytra linearia, apice truncata, leviter dentata vel fere inermia, fere ad apicem punctata, griseo-pubescentia (♂) vel subnuda (♀) guttis binis ante medium, macula transversa pone medium albis vel flavidis maculaque obsoleta grisea apicali ornata. Tarsi breves. Long. corporis 6–8 mm.

NEGROS. SAMAR. SIBUYAN (*Baker*). Baker collection; Riksmuseum, Stockholm.

The two antemedial spots of the elytra are obliquely placed, the interior elongate and somewhat nearer to the base than the exterior.

Daphisia bakeri var. vittulata var. nov.

♂. A forma typica differt vertice flavido-bivittato maculisque elytrorum flavescentibus. Femora testacea; tibiæ et tarsi infuscata.

BASILAN. Baker collection.

Daphisia bakeri var. semisignata var. nov.

A forma typica differt macula antemediana interiore elytrorum deficiente maculaque transversa postmediana majore. Pedes toti testaceo-brunnei.

MINDANAO, Surigao, Surigao. Baker collection.

Daphisia bakeri var. immaculata var. nov.

Elytris unicoloribus immaculatis omnino pube virescente-grisea tectis insignita.

NEGROS. MINDANAO, Lanao, Kolambugan. Baker collection.

SAPERDINI

Genus **PARAZOSNE** novum

Tibiae intermediae integrae. Antennae (♀) corpore breviores, articulis 4–11 opacis. Frons inter oculos constricta.

Tubercula antennifera divergentia sulco angulari separata. Oculi emarginati; lobi inferiores subquadrati. Prothorax cylindricus, ante basin levissime constrictus. Elytra lateraliter costato-deflexa, carinis prope apicem omnino evanescentibus, apice truncata fere inermia. Tibiae apicem versus sensim compresso-dilatatæ. Tarsi breves; articulus primus posticorum 2° et 3° simul sumtis haud longior.

I have been compelled to erect this new genus for the rare and beautiful insect described and figured by Westwood in 1841 as *Colobothea leucospilota* and hitherto referred to the genus *Glenea*, from which it however differs by having the middle tibiæ entire without incision.

The only specimen I have seen is a female and it was taken at Surigao, Mindanao, by Baker. The white markings of the elytra agree rather well with the spots in Westwood's figure.

Parazosne leucospilota Westwood.

Colobothea leucospilota WESTWOOD, Arc. Nat. 1 (1841) 57, pl. 15, fig. 2.

Chalybeate, more or less purple at the sides of the elytra, shining and spotted with white. Front on each side bordered with a fine white line, embracing the outer side of the antennary tubers. Cheeks whitish. Vertex without stripes. Prothorax nearly as long as broad with very few punctures, strongly shining, three small spots above near apical margin and a transverse spot at the base white. Scutellum white, black at base. Elytra very strongly punctured at base and at the sides, shining and without punctures in apical fifth; each with six or seven white markings; a discal dot near base, a short transverse fascia near middle, a lateral dot between the first dot and the fascia, a dot near the suture behind middle, a lateral dot behind the last, an oblique fascia before apex, and often also an apical spot. Body beneath with grayish pubescence along the middle and with white patches on the breast and white apical margins to the first four ventral segments. Femora bluish; tibiæ dark violet; tarsi black. Length, 19 millimeters.

LUZON. MINDANAO.

PHILIPPINE AND MALAYAN PLOIARIINÆ (HEMIPTERA, REDUVIIDÆ)

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FOUR PLATES

The collections reported upon herewith were submitted by C. F. Baker and H. M. Pendlebury. A few specimens from other sources also are included. That there is considerable variety among these insects in the regions concerned is indicated, not only by the diversity of the present collections, but also by the large number of previously described forms, as listed in the bibliography. We regret that we cannot identify a greater proportion of these old species, but the descriptions in most cases fail to mention characters we have found indispensable in classification. Were specimens of these previously established species available for study, however, we have no doubt that most of them would prove distinct from those in hand, as we find a wealth of characters available for segregation of species in the group. We are not so sure that many of the previously described genera would be retained; the natural groups of Ploiariinæ entitled to generic rank are relatively few, in our opinion, and apart from those included in this paper various segregates ranked as genera probably would be better placed as subgenera. The scheme of classification used follows that adopted by the writers for the American Ploiariinæ,[1] with modifications called for by the characters of the material examined.

In accordance with Professor Baker's liberal arrangements, the holotypes of species collected by him and odd-numbered specimens are retained by us, and even-numbered specimens are returned to him. The types from Mr. Pendlebury's material are being deposited in the British Museum, by request of the collector, and only a few duplicates are retained.

[1] Proc. U. S. Nat. Mus. 67 (April, 1925) 1–153, pls. 1–9.

Key to the genera of the Ploiariinæ.

1. Fore tarsus short, normal in form, similar in size and structure to the mid and hind pairs, the segmentation visible under moderate magnification, the claws small and equal; fore tibia four or five times as long as fore tarsus, usually over four-fifths as long as fore femur ..... 2.
   Fore tarsus more or less elongate, longer than mid and hind pairs, and of different structure, heavily chitinized, segmentation visible only under high magnification, or in other cases obsolete, with an unequal pair of claws or a single claw; fore tibia never more than twice as long as, and sometimes shorter than, fore tarsus, usually less than half as long as fore femur..... 8.
2. Fore tibia at least four-fifths as long as fore femur, armature of femur beginning near base..... 3.
   Fore tibia only about half as long as fore femur, armature of femur beginning some distance from base..... **Gardena** Dohrn.
3. Basal sternite deeply angularly emarginate apically (Plate 3, fig. 29); forewing usually with only one closed cell (Plate 3, figs. 23, 30); prothorax only moderately constricted..... 4.
   Basal sternite not deeply angularly emarginate apically; forewing usually with two closed cells (Plate 1, figs. 1, 2; Plate 3, figs. 34, 35); prothorax usually deeply constricted..... 6.
4. Fore tarsi 3-segmented; head, anterior lobe of prothorax, and fore legs uniformly clothed with long hairs..... **Ademula g. nov.**
   Fore tarsi 2-segmented; head, anterior lobe of prothorax, and fore legs not uniformly clothed with long hairs..... 5.
5. Fore femur with tufts of bristles above; forewing with a small closed cell at base of discal cell (Plate 3, fig. 26)..... **Tridemula** Horvath.
   Lacking these characters..... **Empicoris** Wolff.
6. Mesonotum and metanotum without spines; fore tarsi 3-segmented. **Myiophanes** Reuter.
   Metanotum at least with a spine; fore tarsi 2-segmented..... 7.
7. Mesonotum and metanotum each with a spine; prothorax pedunculate. **Stenolemus** Signoret.
   Mesonotum without spine; metanotum and first tergite each usually with a spine; prothorax merely constricted..... **Emesopsis** Uhler.
8. Fore tarsus visibly segmented under high magnification; armature of fore femur extending along practically the whole length of the ventral surface..... 9.
   Fore tarsus unsegmented; armature of fore femur confined to apical half or less of ventral surface..... 10.
9. Pronotum extending over mesonotum to bases of wings; apparent posterior margin of prosternum as seen from below convex in outline. **Bagauda** Bergroth.
   Pronotum not extending over mesonotum except at its apex, falling much short of bases of wings; apparent posterior margin of prosternum as seen from below usually depressed medianly, appearing emarginate..... **Ploiaria** Scopoli.
10. Fore tarsus including claws shorter than fore tibia, the claws paired, unequal, short and curved; third antennal segment about one-tenth as long as fourth; head longer behind than in front of eyes..... 11.

Fore tarsus including claw longer than fore tibia, the claw single, nearly straight, and about one-third as long as tarsus (Plate 4, fig. 55); third antennal segment about half as long as fourth; head shorter behind than in front of eyes..... **Phryxobotrys g. nov.**

11. Metathorax much shorter than mesothorax..... **Ischnonyctes Stål.**
Metathorax little if any shorter than mesothorax..... **Ischnobaena Stål.**

Genus EMESOPSIS Uhler

The oriental fauna teaches us in this case, as in many others, that we must take a broad view of genera, or envisage the erection of an indefinite additional number of them. At first glance the reticulate-veined base of the forewing, as exemplified by the genotype of *Emesopsis,* would seem distinctive; but in the present collection there are species in which one can scarcely decide whether the reticulation really consists of veinlets or is merely color pattern. There are various styles of this reënforcement of the base of the wing, varying from broadened basal veins (as in *E. velutinervis,* Plate 1, fig. 9) to the narrow strip of minute reticulations of species like *E. neptunis* (Plate 1, fig. 1), and the definitely cross-veined condition of the genotype (*E. nubilus* Uhler). We see no grounds for generic distinctions in this material.

Calphurnia Distant,[2] although described as having the anterior tarsi 3-segmented, possibly is a synonym of *Emesopsis.* The same is true of *Calphurnioides* Distant.[3] *Bironiola* Horvath[4] also is not far removed.

Key to the subgenera and species of Emesopsis Uhler.

1. A small closed cell at inner anterior angle of discal cell of forewing (Plate 1, figs. 1 to 3); head and thorax copiously pubescent; armature of fore femur bristly, without spines..... 2.
No small closed cell at inner anterior angle of discal cell of forewing (Plate 1, fig. 4); head and thorax sparsely haired; mid and hind femora somewhat clavate at both extremities, a single prominent spine near base, and a few spinules scattered along ventral surface of fore femur (*Pseudobolos* subg. nov.; subgenotype, *velutinervis* sp. nov.)..... 8.

2. A stump of a vein emanating from apex of discal cell in addition to the vein that runs toward apex of wing, the latter vein with a branch toward costal margin which is bifurcate, the rami evanescing; forewing with ocellate spots, but lacking cross veinlets basad of small discal cell (Plate 1, fig. 1); dense pile of anterior lobe of pronotum interrupted by a glossy trident-shaped mark on each side (subg. *Hadrocranella* Horvath)..... 3.

[2] Ann. Mag. Nat. Hist. VIII 3 (1909) 502.
[3] Trans. Linn. Soc. London II 16 (1913) 164.
[4] Ann. Mus. Nat. Hung. 12 (1914) 639–640.

No stump of a vein at apex of discal cell; apical vein simple; forewing without ocellate spots; pronotum without trident-shaped bare areas; both costal and discal areas near base of wing with transverse veinlets or more or less veinlike dark markings (subg. *Emesopsis* Uhler) ..... 4.

3. Small closed cell at base of discal cell distinct; wing basad of it with a very narrow strip of dark reticulations and without dark transverse lines (Plate 1, fig. 1)..... **E. (H.) neptunis** sp. nov.
   Small closed cell almost obsolete; wing basad of it with a broader stripe of dark reticulations and with a few dark transverse lines joining costa (Plate 1, fig. 2)..... **E. (H.) obsoletus** sp. nov.
4. Apical process of male hypopygium spinelike, the apices of claspers almost rectangularly upcurved (Plate 1, figs. 13, 14). **E. (E.) spicatus** sp. nov.
   Apical process of male hypopygium broad, more or less scooplike, that is, the posterior surface distinctly concave (Plate 1, fig. 15); claspers little if any upcurved..... 5.
5. Discal cell of forewing with a subquadrate dark spot notably larger than its fellows, at bend of the vein closing its apex on anterior half (Plate 1, fig. 6); ædeagus with the dorsal hooks broad and strong (Plate 2, figs. 19, 20)..... 6.
   Discal cell of forewing with the dark spots along veins closing its apex subequal in size (Plate 1, fig. 5); ædeagus with the dorsal hooks slender (Plate 2, figs. 21, 22)..... 7.
6. Hypopygium of male as in Plate 2, fig. 19..... **E. (E.) gaius** sp. nov.
   Hypopygium of male as in Plate 2, fig. 20..... **E. (E.) gallienus** sp. nov.
7. Hypopygium of male as in Plate 2, fig. 21..... **E. (E.) nero** sp. nov.
   Hypopygium of male as in Plate 2, fig. 22..... **E. (E.) hadrian** sp. nov.
8. Mid and hind femora slightly thickened at bases and apices, slenderer mesially, with three slight beadlike swellings, one before and one beyond middle, the other just before apex; hypopygium of male large, the apical spine thick, tapered to a sharp point; not much curved upward, and extending beyond apices of claspers (Plate 1, fig. 16) ..... **E. (P.) moniliferus** sp. nov.
   Mid and hind femora lacking beadlike swellings; apical spine of male hypopygium more or less erect, shorter than claspers..... 9.
9. Posterior lobe of pronotum faintly rugose; longitudinal veins of basal part of forewing deep velvety black, not connected by transverse markings or veinlets (Plate 1, fig. 9)..... **E. (P.) velutinervis** sp. nov.
   Posterior lobe of pronotum rather coarsely rugose; longitudinal veins of basal part of forewing not so deep black, connected by a dark marking, or by a thickening of the membrane..... 10.
10. Only one connection between longitudinal veins of basal part of forewing (Plate 1, fig. 8)..... **E. (P.) emmesius** sp. nov.
    Two connecting marks or veinlets between longitudinal veins of basal part of forewing (Plate 1, fig. 7)..... **E. (P.) connexus** sp. nov.

Emesopsis (Hadrocranella) neptunis sp. nov.

Male and female.—Head and body testaceous, the head and pronotum covered with dense gray pile and bearing sparse long hairs also, the venter with short sericeous pubescence. An-

tennæ stramineous, first segment with a dark annulus near base, and traces of one subapically; appressed pubescent, rather long-haired basally on first segment. Fore coxæ and mid and hind femora and bases of tibiæ with copious long spreading hairs, most of which are about six times as long as diameter of parts on which they are situated; mid and hind femora with brownish traces of subapical annuli. Fore femur pale brownish, with median, subapical, and apical, whitish annuli (Plate 1, fig. 10); fore tibia chiefly pale, with a subbasal and an apical brownish annulus. Forewing whitish hyaline, the veins yellowish to brownish, that from the small closed cell toward base of wing paralleled on each side by a narrow strip of fine brownish reticulations; stigma with a dark dot near base; interior of large discal cell with irregular blackish irroration; a conspicuous round black spot just posterior to discal cell and a larger, irregularly quadrate spot on each margin of wing behind that; apex of wing with two or three dusky spots; veins in apical half of wing margined with dusky (Plate 1, fig. 1).

Basal segment of antenna and mid and hind femora slightly clavate apically. Mesonotum rather normally scutellumlike, metanotum and first tergite each with a prominent spinelike tubercle. Abdomen gradually widened from base to near apex; hypopygium and apex of last tergite of male as in Plate 2, fig. 17.

Length, 5 to 6 millimeters.

Holotype male, allotype, and 3 male paratypes, Surigao, Mindanao; paratype, Mount Maquiling, Luzon (*Baker*).

This species is allied to *Ploiariodes medusa* Kirkaldy[5] but apparently is distinct.

So far as we can judge from the description and figure, *Hadrocranella imbellis* Horvath[6] is so closely related to *medusa* and *neptunis* that its generic name may properly be used for the group we regard as a subgenus of *Emesopsis*.

Emesopsis (Hadrocranella) obsoletus sp. nov.

Male.—Hairs much less conspicuous than in *E. neptunis* except on fore coxæ. Antennæ stramineous, first segment with a broad subbasal, a narrow median, and a subapical narrow annulus, second segment with a broad subbasal and a narrow apical annulus, fuscous; third segment darkened near base and on the apical half, and fourth at base and apex. Mid and hind femora

[5] Proc. Linn. Soc. N. S. W. 33 (1908) 373, pl. 4, fig. 12 (Fiji).
[6] Ann. Mus. Nat. Hung. 12 (1914) 647–649, fig. 8 (New Guinea).

with three widely spaced dark annuli, and tibiæ with two near base; fore coxa broadly fuscous at apex, and with a narrow fuscous median annulation, in structure slenderer basally than in *E. neptunis;* fore femur with a broad subbasal, a broad subapical, and a narrow median annulus, fuscous, the subbasal annulus pale in center (Plate 1, fig. 11); fore tibia with a narrow and a broader fuscous annulation near base, and the apex fuscous. Forewing pattern much as in *E. neptunis*, with the differences described in key, and stigma with a conspicuous black spot apically; large quadrate spot beyond apex of discal cell on inner side of wing divided into two (Plate 1, fig. 2). Abdomen testaceous, rather conspicuously blackened on apical third except tip, much more strikingly attenuated basally and more abruptly widened from middle to near apex than in *neptunis;* hypopygial claspers and apical process shaped much as in *hadrian*. Scutellum not elongate triangular as in the other species of the subgenus, but broadly rounded in posterior outline and with a blunt convexity centrally.

Length, 5 millimeters.

SINGAPORE, holotype and paratype (*Baker*).

This species is even more closely similar than *E. neptunis* to *Ploiariodes medusa* Kirkaldy, but there are some points of difference (as *medusa* having two dark annuli close to base of each mid and hind femur), and geographic considerations lead us to believe that with the specimens in hand other distinctions could readily be found.

Emesopsis (Emesopsis) spicatus sp. nov.

Male.—General color of head and thorax fuscous, with grayish flocculose pubescence; antennæ and mid and hind legs testaceous, with spreading hairs basally and appressed pubescence apically. Antennæ with indistinct darker annulations; fore coxa with a single subapical fuscous annulus; fore femora much stouter than in the two preceding species (Plate 1, fig. 12), with five faint fuscous annuli; mid and hind legs without dark annulations, the tibiæ somewhat darker than femora, the latter slightly clavate, surface hairs not or but slightly longer than femoral diameter. Forewing with dark lines in both costal and radial areas basad of discal cell; remainder of wing with irregular dusky blotches. Hypopygium as in Plate 1, figs. 13, 14; Plate 2, fig. 18.

Length, 6 millimeters.

SIBUYAN, holotype (*Baker*).

Emesopsis (Emesopsis) gaius sp. nov.

Male and female.—The general color of head and body testaceous, of appendages stramineous, the former covered with close, crisped, gray pubescence, the latter with somewhat longer, straighter, and mostly appressed hairs. Subbasal annulus on first segment of antenna and extreme base of second segment fuscous. Fore legs structurally as in *E. spicatus*, with indistinct brownish annulations, a subapical one on coxa, three or four on femur and on tibia; mid and hind femora with three or four narrow dark annuli, the dark parts slightly thickened; tibiæ of these legs in general darker than the femora, sometimes with two or three faint dark annuli. Transverse dark lines on basal third of forewing distinct, a large dusky blotch on each margin opposite small discal cell, remainder of wing with numerous irregular dusky spots (Plate 1, figs. 3, 6). Male hypopygium as in Plate 2, fig. 19.

Length, 6 millimeters.

SINGAPORE, holotype, male allotype, 1 male and 4 male paratypes (*Baker*).

Emesopsis (Emesopsis) gallienus sp. nov.

Male.—Coloration much as in *E. gaius;* fore femur and tibia each with four fuscous annulations. Wing markings less distinct. Hypopygium as in Plate 2, fig. 20.

Length, 6 millimeters.

PALAWAN, Puerto Princesa, holotype and paratype (*Baker*).

Emesopsis (Emesopsis) nero sp. nov.

Male and female.—Ground color of body testaceous, of appendages stramineous; gray vestiture of former rather woolly, of latter mostly appressed. Basal segment of antenna with several narrow brownish annuli, much more conspicuous on under than on upper side. Fore coxa with a subapical brown annulus, more conspicuous on inner side; fore femur with about four faintly indicated annuli on outer side, and a continuous brown longitudinal stripe on the inner; fore tibia brownish at base and apex. Mid and hind femora each with four faint brownish annulations. Forewings whitish hyaline, markings pale brownish; veins costad of small discal cell, and a small spot at apex of stigma darker; spots in discal cell as in Plate 1, fig. 5. Male hypopygium as in Plate 1, fig. 15; Plate 2, fig. 21.

Length, 6 millimeters.

Holotype, male, allotype, 1 male and 3 female paratypes, Mount Maquiling, Luzon; 2 male and 2 female paratypes, Basilan (*Baker*).

Emesopsis (Emesopsis) hadrian sp. nov.

Male.—Coloration as in *E. nero*, but the dark markings, especially of the appendages, fainter. Male hypopygium as in Plate 2, fig. 22.

Length, 6 millimeters.

LUZON, Mount Maquiling, holotype (*Baker*).

Emesopsis (Pseudobolos) moniliferus sp. nov.

Male.—General color testaceous, eyes, posterior lobe of pronotum, bases of forewings, and underside of mesothorax blackish. Pubescence of pronotum longer than on other parts of the insect, but scarcely woolly; that of abdomen and appendages mostly appressed. Basal segment of antenna with the faintest indications of brownish annuli, the "beads" of mid and hind femora washed with brownish. Forewings hyaline, veins near base of the long stigma blackish.

Metanotum with a long, slender, slightly recurved, acute, and pale spine; mesonotum and first tergite rounded tuberculate. Hypopygium as in Plate 1, fig. 16.

Length, 7 millimeters.

MINDANAO, Zamboanga, Dapitan, holotype (*Baker*).

Emesopsis (Pseudobolos) velutinervis sp. nov.

Male and female.—General color testaceous, eyes and posterior lobe of pronotum blackish. Antennæ and legs without dark annulations; segments 2 to 4 of antennæ sometimes blackish. Forewings whitish hyaline, two longitudinal vittæ over veins in basal half of wing and one or two in discal cell velvety black (Plate 1, fig. 9); narrow markings paralleling other veins and margins, two curved streaks in subapical part of wing, and a series of wedge-shaped blotches about apex, dusky.

Length, 4.5 to 5 millimeters.

Holotype, male, and allotype, Mount Maquiling, Luzon; 1 male and 1 female paratypes, Dapitan, Mindanao (*Baker*).

Emesopsis (Pseudobolos) connexus sp. nov.

Male and female.—Coloration almost exactly as in *E. velutinervis* (except for that of the forewing which differs as stated in key and shown in Plate 1, fig. 7) and in having irregular

blotches instead of regularly arranged wedge-shaped spots at apex. The posterior lobe of pronotum also tends to be less extensively blackened.

Length, 5 to 6 millimeters.

BORNEO, Sandakan, holotype, male, allotype, 1 male and 1 female paratypes (*Baker*).

Emesopsis (Pseudobolos) emmesius sp. nov.

Female.—General coloration as in *E. connexus;* forewing differing as stated in key, and illustrated in Plate 1, figs. 4 and 8, fore legs somewhat darker, the femur with a more or less distinct median pale annulus.

Length, 6 millimeters.

Holotype, Sibuyan; paratype, Basilan (*Baker*).

Genus **ADEMULA** novum

Closely similar to *Empicoris*, differing essentially in having the fore tarsi 3-segmented. In both species of the genus before us the stigma of forewing is carried much nearer to apex of wing than is the case in any species of *Empicoris* or *Tridemula* we have seen, and the lateral pronotal carina is incomplete, which is only exceptionally the case in *Empicoris*. Mesonotum and first tergite each with a spine; metanotum only tuberculate.

Genotype, *Ademula reticulata* sp. nov.

Key to the species of Ademula g. nov.

1. Dark markings of forewing with fine reticulating white lines intersecting them (Plate 3, fig. 23); hind lobe of pronotum little if any darker than front lobe; fore femora very densely haired. **A. reticulata sp. nov.**

   Dark markings of forewings not intersected by reticulating lines; hind lobe of pronotum dark fuscous, anterior lobe testaceous; fore femora less densely haired..... **A. nubecula sp. nov.**

Ademula reticulata sp. nov.

Male and female.—General color stramineous, head, basal segment of antenna, anterior lobe of pronotum, and front legs with copious long hairs. Basal segment of antenna with two narrow dusky annulations near apex. Posterior lobe of pronotum with three more or less evident brown vittæ, anterior lobe of prothorax and posterior lobe of head in some cases darker medially. Forewing stramineous, with a practically continuous dusky vitta from near base to apex, this vitta with pale reticulating lines and spots; stigma with a faint brown spot at middle (Plate 3,

fig. 23). Hind wings unspotted. Mid and hind legs with a few very narrow annulations varying from faint brown to black, those near femorotibial joints the most noticeable. Fore femur with four, fore tibia with three, brown annuli, the hairs on which are darker than those of the adjacent pale areas. Venter sericeous with fine pale down.

The armature of the fore femur consists of a series of from three to five stout spines on the posteroventral surface, which are about half as long as the femoral diameter and extend from near base to beyond middle, and a series of short pale peglike spines on almost the entire length of ventral surface, with one or two longer ones situated at basal extremity of the series on the anteroventral surface, evidently to act as a rest for the opposing tarsus. Basal segment of antennæ and mid and hind femora slightly clavate. Hypopygium of male with a stout apical process which projects upward between the claspers and always separates them, the tip of the process more or less tumid (Plate 3, fig. 24).

Length, 5.5 to 6.5 millimeters.

Holotype, male, allotype, 1 male and 2 female paratypes, Singapore; paratypes, Sandakan, Borneo (*Baker*).

Specimens from the Philippines generally paler and with the wing markings especially reduced may be known as var. *abluta* var. nov. Holotype, female, Mount Limay, Luzon; paratypes Mount Limay, Mount Maquiling, Luzon; Dapitan, Mindanao (*Baker*); Kuala Lumpur, F. M. S., October 24, 1923, at light (*H. M. Pendlebury*).

Ademula nubecula sp. nov.

Male.—General color pale fuscous, hind lobe of head and front lobe of pronotum testaceous; antennæ, beak, and fore legs testaceous, the femora with four, the tibiæ with three, indistinct fuscous annuli; mid and hind legs stramineous, with touches of fuscous, especially near femorotibial joints. Pubescence much as in *A. reticulata*. Forewings whitish hyaline, with a nearly percurrent vitta, which is dusky bluish, without intersecting pale reticulating lines; the veins in the vitta are darker than those outside; pale areas modifying the shape of the vitta are at the base of the discal cell and at about the middle of the cell, another opposite latter on inner margin of the wing and at the wing apex, most of which is pale.

Mid and hind femora slightly, basal segment of antenna scarcely, clavate apically. Fourth antennal segment one-third as long as third. Armature of fore femur as in *A. reticulata*. Hypopygium with a much slenderer, erect, apical process hidden by the apices of claspers which meet on median line (Plate 3, fig. 25).

Length, 5.5 millimeters.

BORNEO, Sandakan, holotype (*Baker*).

Genus **TRIDEMULA** Horvath

The presence of a closed cell basad of the discal cell in forewing (Plate 3, fig. 26) and of four tufts of bristles on dorsal surface of fore femur, readily distinguishes this genus from *Empicoris*, to which it is most closely related. Both genera have the posterior lobe of pronotum more or less carinate laterally, and both have the basal abdominal sternite angularly emarginate apically, though in *Empicoris* the emargination is much less pronounced. There is a striking difference between the hypopygia of the two species of *Tridemula* here described (Plate 3, figs. 27, 28), but the same variation is found in *Empicoris*, most of the species with the apex deeply emarginate occurring in the Orient. Mesonotum and metanotum each with a spine, first tergite no more than tuberculate.

Genotype, *Tridemula pilosa* Horvath.

Key to the species of Tridemula Horvath.

1. Hind lobe of pronotum with a pale elliptical tubercle on middle of hind margin; stigma of forewing with a red line along its inner margin apically..... **T. plurima sp. nov.**
   Hind lobe of pronotum without a tubercle above; stigma without a red line..... **T. pallida sp. nov.**

Tridemula plurima sp. nov.

Male and female.—General color testaceous, basal two segments of beak and eyes blackish. Head with very short pale sericeous pubescence, first segment of antenna with sparse, long, spreading hairs, and remaining segments with shorter, more appressed hairs. Thorax with copious, short silvery pubescence and sparse long hairs, the pronotal tubercle somewhat paler than the surrounding surface. Forewing whitish hyaline, dusky maculate, spots at base and apex of stigma blackish; venation as in Plate 3, fig. 26. Hind wings unspotted. Mid

and hind legs whitish, a faint brownish band near apex of each femur. Fore coxa and femur with copious hairs of moderate length, and the femur with four, about equidistant, transverse tufts of blackish bristly hairs on its dorsal surface, 2 and 3 less conspicuous than 1 and 4, a broad annulus from tuft 1 to 3 and base and apex of femur pale; front tibia with two pale annuli near base. Venter with very short, pale, sericeous pubescence.

Pronotal tubercle elongate, elliptical as seen from above. None of the segments of legs and antennæ clavate. Venter of thorax and basal sternite as in Plate 3, fig. 29; penultimate sternite with a small rounded central apical lobe; ultimate sternite with a shallow rounded central apical emargination; apex of hypopygium bispinose (Plate 3, fig. 27). The armature of the fore femur consists of two series of short stubby spines, one anteroventral, the other posteroventral, with longer spines as in *Ademula* species.

Length, 6 millimeters.

Holotype, male, and allotype, Singapore; paratypes, male, Mount Maquiling, Luzon; female, Puerto Princesa, Palawan (*Baker*).

Tridemula pallida sp. nov.

Male.—Similar to *T. plurima* in vestiture and coloration, but paler. General color stramineous, eyes black, a dark vitta on head behind eye, stigmal spots less conspicuous than in *T. plurima*. Mid and hind legs with very faintly indicated dusky annuli, fore legs colored as in *T. plurima*.

Fore femur as well as the abdomen as in *T. plurima* except for hypopygium (Plate 3, fig. 28).

Length, 6 millimeters.

SINGAPORE, holotype (*Baker*).

Genus EMPICORIS Wolff

Mesonotum, metanotum, and first tergite each usually with a spine.

Key to the species of Empicoris Wolff.

1. Hind lobe of pronotum with a tubercle on middle of hind margin..... 2.
   Hind lobe of pronotum without a tubercle as above..... 3.
2. Pronotal tubercle small and inconspicuous, most easily seen from posterior view; posterior pronotal lobe brown, with lateral carinæ, hind margin, and two discal lines silvery white..... E. bilineatus sp. nov.
   Pronotal tubercle large and conspicuous; pronotal lobe with the pale discal lines very faint, or lacking..... 3.

3. Lateral carina of pronotum decidedly angulate about one-third from anterior end; length of forewing 3.5 millimeters; margin of hypopygium produced centrally, tip of process slightly emarginate (Plate 3, fig. 31)..... **E. bakeri** sp. nov.

Lateral carina of pronotum merely curved; length of forewing 5 millimeters; margin of hypopygium broadly excavated (Plate 3, fig. 32). **E. discalis** sp. nov.

4. Lateral carina of hind lobe of pronotum distinct only at anterior and posterior extremities and white only at former; apical black band on mid and hind femora falling distinctly short of apices of femora.... 5.

Lateral carinæ entire and all white, apical black band on mid and hind femora broad, extending to, or almost to, apices of femora..... 6.

5. A bright red streak at inner apex of stigma. **E. rubromaculatus** (Blackburn).

Stigma not partly red. **E. rubromaculatus** var. **obsoletus** McAtee and Malloch.

6. Pigmentation more pronounced, discal cell of forewing densely maculate. **E. tessellatus** sp. nov.

Pigmentation less pronounced, discal cell of forewing chiefly hyaline. **E. lavatus** sp. nov.

Empicoris bilineatus sp. nov.

Described from a specimen with head and most of abdomen missing. Pronotum as described in key; forewings with numerous dusky blotches, leaving between them a whitish reticulation, three larger, more pronounced blotches on outer margin near apex, and a series of regularly spaced ones on inner margin, which decrease gradually in size from apex toward clavus, stigma with one basal and two apical black spots, venation about discal cell as in Plate 3, fig. 30; hind wings unspotted. Legs whitish, with narrow black annulations, the front leg with five on femur and four on tibia.

Carina on side of pronotum continuous, with a very short peglike process anteriorly; mesonotum and metanotum each with a short, slender, nearly erect spine; first abdominal tergite with a much longer, posteriorly curved, and slightly clavate process; mid and hind femora slightly clavate apically.

Length of forewing, 3 millimeters.

LUZON, Laguna, Mount Maquiling, holotype (*Baker*).

Empicoris bakeri sp. nov.

Male.—Head blackish brown, with silvery hairs, a definite band of which margins orbit; antenna moderately hairy, whitish, with numerous dark annulations. Pronotum brown, with the lateral carinæ and other lines of hairs silvery white, the median pair on posterior lobe not so well defined as in *E. bilineatus*.

Forewing marked much as in that species, with several large clear areas, however, and the spots at posterior end of stigma confluent; hind wing unspotted. Legs whitish, the posterior two pairs ornamented with alternating narrower and wider annulations; the fore legs with the dark markings more massed near ends of the segments.

Eye large, notably wider than interocular space; antenna half again as long as body, first segment slightly clavate apically. Lateral carina of pronotum clear-cut, distinctly elevated, its anterior extremity, however, scarcely produced; median tubercle of posterior lobe prominent; mesonotal and metanotal spines fully twice as long as in *E. bilineatus*, slender, directed upward and backward. Spine on first tergite like that of *E. bilineatus;* hypopygium from behind as in Plate 3, fig. 31. Mid and hind femora longer than body, scarcely clavate.

Length, 5 millimeters.

LUZON, Laguna, Mount Maquiling, holotype (*Baker*).

Empicoris discalis sp. nov.

Male.—A larger, more contrastingly marked species than *E. bakeri*, the ground color brownish black. Head with short pale hairs scarcely aggregated in lines. Antennæ and mid and hind legs appressed pubescent, with alternating dark annulations and spots. Fore leg with basal two-thirds of coxa, apex and two subapical bands on femur, and four annulations on tibia, the distal one widest, whitish. Pronotum with margin of posterior lobe, carinæ, and lines of hairs on anterior lobe silvery white; a large quadrate white spot on anterior disk of posterior lobe, prolonged anteriorly into the groove of anterior lobe. Markings of forewing much as in *E. bakeri*—that is, more conspicuous about the margins than discally, except near base; there is a large black spot on costa near base of discal cell, a small one at base, and a large one toward apex of stigma; hind wings unspotted.

Basal segment of antenna, and mid and hind femora scarcely clavate. Hypopygium from behind as in Plate 3, fig. 32.

Length, 6 millimeters.

Holotype, Jor Camp, Perak, F. M. S., March 10, 1924, at light (*Pendlebury*).

Empicoris lavatus sp. nov.

Male.—General color of head and body castaneous, head with pale hairs, which are more or less aggregated along orbits, be-

hind transverse impression, and about bases of antennæ; antenna whitish with dark bands, the bands on first segment narrower than, those on other segments about equal to, intervals, first segment with long spreading hairs, others with short, more appressed hairs. Disk of pronotum pale, anterior lobe, sides, and lateral carina of posterior lobe with lines of silvery hairs. Forewings sordid whitish, with mostly faint brownish blotches, the discal cell largely unmarked; stigma with one basal and two subapical brown spots; a larger solid brown spot on costa just beyond stigma. Hind wings unspotted. Venter with short silvery hairs. Legs whitish, with narrow, well-separated dark annulations, mid and hind femora with broader and darker terminal bands; fore femur dark, with two narrow pale annuli near middle.

Antennæ and mid and hind legs much longer than body, none of their segments clavate. Lateral carina of pronotum sharp and distinct throughout without process anteriorly, disk of posterior lobe with two low longitudinal swellings; hind margin trisinuate, without tubercle. Mesonotal and metanotal spines moderately developed, first tergite with a slender spine.

Length, 5 millimeters.

Holotype, Mount Maquiling, Luzon; paratype, Surigao, Mindanao (*Baker*).

Empicoris tessellatus sp. nov.

Female.—Agrees in most respects with *E. lavatus*, but decidedly more pigmented, especially the forewings. Basal segment of antenna without long, spreading hairs. Forewing densely maculate throughout, two spots in middle of discal cell notably larger than the others; stigma nearly covered by confluent dark spots basally, pale apically. Hind wings unspotted. Fore femur and tibia nearly black, the former with two, the latter with three, narrow pale bands.

Mesonotal and metanotal spines as in *E. lavatus*, the first tergite also with a slender spine which is slightly curved posteriorly.

Length, 5 millimeters.

SINGAPORE, holotype (*Baker*).

Empicoris rubromaculatus (Blackburn).

Ploiariodes rubromaculatus BLACKBURN, Proc. Linn. Soc. N. S. W. II 3 (1889) 349 (Hawaii).

A specimen from Mount Maquiling, Luzon (*Baker*), although somewhat heavily pigmented and having the median spines rather feebly developed, seems referable to this cosmopolitan species. A female specimen from the same locality, and a male from Dapitan, Mindanao (*Baker*), may be grouped with that from Funchal, Madeira (holotype), mentioned in our paper on the American Ploiariinæ (1925, p. 17) as *Empicoris rubromaculatus* var. *obsoletus* var. nov., characterized by the absence of a red streak in the stigma.

Genus **STENOLEMUS** Signoret

Key to the species of Stenolemus Signoret.

1. Hind femur with four narrow black bands, the basal two with dense brushlike tufts of black hairs (Plate 3, fig. 33); mid tibia with a small black spot close to base above and a black ring a little beyond it, the latter with long dense brushlike hairs; hind tibia without the subbasal black spot, the band broader and with longer hairs than midtibial band, and with a much narrower band with shorter hairs between it and middle of tibia..... S. plumosus Stål.

   Hind femur with four brown bands which are broader than the intervening white spaces, none of them with more conspicuous hairs than remainder of femur; mid and hind tibiæ brown, with three broad white bands, one at base and the apical one not much beyond middle, none of the dark bands conspicuously haired..... S. quadriannulatus sp. nov.

Stenolemus plumosus Stål.

Stenolemus plumosus STÅL, Öfv. Kgl. Vet.-Akad. Förh. **27** (1870) 702 (Philippines).

Female.—Head brownish fuscous, with gray downy hairs, which extend over basal segment of beak; antenna white, the basal segment with long, the others with numerous shorter pale hairs, basal segment with five fuscous annuli; second with four, which become progressively wider from base to apex, third yellow only at apex, fourth mostly dark, pale just before apex; each segment of beak broadly brown at base. Pronotum with a central pale line and two oblique, posteriorly convergent, pale lines on each side of anterior lobe, the lateral lines merging into one which extends along dorsum of petiole, posterior lobe largely brown, paler on each anterior lateral angle and with three faint paler dorsal vittæ; mesonotal and metanotal thorns dark brown, the latter pale at tip. All parts of thorax with sparse long spreading hairs, and copious surface down, pale. Abdominal venter brownish fuscous, with indications of three slender pale vittæ, and paler lateral margins to segments, the

elevated spiracles and lateral angles of segments darker; dorsum mostly dark brown. Legs white; fore legs with the following brown annuli: Coxæ, 2; femora, 4; tibiæ, 5; tarsi, 1. Mid legs annulate as follows: Femora, 4, all narrow, and a spot at apex above; tibiæ, 2, and a basal spot, the basal annulus furnished with dense black hairs forming a brush which is much deeper than diameter of tibia; apices of tibiæ, and the tarsi fuscous; hind legs annulate like the mid pair, but the two basal annuli on femora densely black-haired while only one is haired on mid legs and that only slightly, and the tibiæ have an additional black-haired annulus nearer middle, while the tuft of hairs on basal annulus is even more conspicuous than on mid pair (Plate 3, fig. 33); tip of tibia and the tarsus fuscous. Forewings chocolate brown, veins paler, some sections and a few short costal streaks as well as some reticulating marks near apex white, the discal cells with faint pale reticulations; hind wings fuscous.

Head across eyes wider than anterior lobe of pronotum; posterior lobe of head with two prominent sharp dorsal tubercles; beak stout; third segment of antenna one-third as long as fourth. Fore legs normal, the basal ventral spine sloped backward. Anterior lobe of prothorax with a rounded, polished tubercle on each side, petiole about 1.5 times as long as posterior lobe, the latter with four prominent sharp tubercles near hind margin; metanotal spine erect, about twice as thick as the horizontal mesonotal one, moderately hairy, almost straight, bluntly rounded at apex. Venter of abdominal segments 3 to 5 with the spiracles tuberculate, the segments angulate on the sides posteriorly. A short oblique vein emitted from near base of basal discal cell connects with costa, transverse vein to claval vein at middle of basal discal cell, posterior discal cell about 1.5 times as long as basal one; hind margin of apex of forewing slightly emarginate.

Length, 14 millimeters.

MINDANAO, Surigao, Surigao (*Baker*).

Stenolemus quadriannulatus sp. nov.

Male.—Paler than the preceding species, the dorsum of head with two pale vittæ uniting behind; petiole of pronotum pale, brownish on sides, posterior lobe yellowish white, with or without faint brownish vittæ, head and pronotum with longer sparse hairs and shorter dense pale reddish pubescence; veins of fore-

wings more conspicuously white than in *S. plumosus*, and the legs differing as stated in key; antennæ and legs moderately hairy, the latter without tufts.

Posterior lobe of head slightly depressed in middle anteriorly. Anterior lobe of pronotum with prominent "shoulders," each with a small domelike polished area; posterior lobe of pronotum with the four tubercles smaller and blunter than in the last species, the mesonotal and metanotal thorns slenderer, subparallel, projecting upward and backward, and slightly recurved. Venation of forewing similar to that of last species, but the posterior discal cell is more acutely pointed at apex, and the transverse vein connecting with anal vein is not over one-fourth from apex of basal discal cell. Venter colored as in preceding species, spiracles 3 to 5 less prominently tuberculate, and sides of segments scarcely angulate.

Length, 12 to 13 millimeters.

MINDANAO, Surigao, Surigao, holotype and paratype (*Baker*).

Stenolemus crassirostris Stål,[1] described from the Philippines, is smaller than either of the preceding species, has the peduncle of pronotum shorter than anterior lobe, and the beak distinctly thickened.

Genus **MYIOPHANES** Reuter

The species here described differ from previously described *Myiophanes* in having the forewings heavily pigmented, obliquely truncate, and somewhat emarginate on the inner half of apex, as in various species of *Stenolemus*. They agree with *Stenolemus* also in having two large closed discal cells in the forewing, and in having the mid and hind legs long, slender, long-haired, and annulate, and the fore femora spined beneath for their entire length. However, as in other species of *Myiophanes*, the anterior lobe of the pronotum is contracted and narrower than the posterior lobe, but not pedunculate as in *Stenolemus*. The fore femur has two series of spines and the fore tibia one series on ventral surface, about every fourth spine longer than the others, the basal posteroventral spine on femur not bent basad, and the anteroventral series not extending to base and without isolated basal spine. The 3-segmented tarsi of the fore legs and the absence of mesonotal and metanotal spines also distinguish the genus from *Stenolemus*.

Genotype, *M. tipulina* Reuter.

[1] Öfv. Kgl. Vet-Akad. Förh. 27 (1870) 702, 703.

Key to the species of Myiophanes Reuter.

1. Mid and hind femora stramineous, each with three broad dark brown bands which together occupy about half of the surface; the tibiæ pale, each with a narrow subbasal brown annulus; forewing as in Plate 3, fig. 35..... **M. annulifera sp. nov.**

   Mid and hind legs stramineous, the femorotibial region broadly whitish, bounded on each side by a narrow brown annulus; forewing as in Plate 3, fig. 34..... **M. fluitaria sp. nov.**

Myiophanes annulifera sp. nov.

Female.—General color stramineous, the basal segment of antenna, the fore coxæ and femora, the hind legs, and the abdomen with moderately long spreading pale hairs. First segment of antenna brownish near base and apex; fore femora with three brownish annuli coalescent in a stripe on upper surface, also brownish apically; fore tibia with a subbasal annulus, and the apex brown; mid and hind legs as described in key. Pronotum with two broad lateral vittæ, and a long triangular mark with its base on the posterior margin, attenuate anteriorly, reaching nearly to middle of anterior lobe. Markings of forewing as in Plate 3, fig. 35.

Dorsum of head with a pronounced rounded elevation on middle of anterior margin of section behind the transverse depression, which projects slightly over the depression.

Length, about 15 millimeters.

Holotype, a dismembered specimen from Jor Camp, about 540 meters, Balang Padang, F. M. S., June 3, 1923 (*Pendlebury*).

Myiophanes fluitaria sp. nov.

Male.—General color stramineous, hairiness much as in *M. annulifera*, that on antennæ less conspicuous, that on legs more so; pronotum also long-haired. Antennæ scarcely annulate, the distal segments darker than the basal. Fore coxa with two, and fore femur with three, broad brownish annuli; fore tibia brownish except at base. Mid and hind legs as described in key. Pronotum colored much as in *M. annulifera*, the median vitta prolonged over anterior lobe, where it is expanded and interrupted by three narrow pale streaks. Coloration of forewing as in Plate 3, fig. 34.

No pronounced elevation behind transverse depression on dorsum of head. Apex of hypopygium with a long spike which is directed backward and curves upward, the claspers long, curved, slightly broadened near apices.

Length, 23 millimeters; of hind femur, 25; of hind tibia, 40.

Holotype, Kuala Lumpur, F. M. S., Gombak Valley, at light, October 13, 1921 (*Pendlebury*).

Genus GARDENA Dohrn

Key to the species of Gardena Dohrn.

1. Basal and apical unspined sections (that is, without long spines) of fore femora about equal in length, each a little less than half as long as the spined median section; head more than half as long as anterior lobe of prothorax..... **G. brevicollis** Stål.

   Basal unspined portion of fore femora almost or fully twice as long as the apical unspined portion, and but little or not at all shorter than the median spined portion; head distinctly less than half as long as anterior lobe of prothorax..... 2.

2. Fore femur without pale preapical annulus.
   G. melinarthrum var. melinarthrum Dohrn.

   Fore femur with conspicuous pale yellow preapical annulus.
   G. melinarthrum var. femoralis var. nov.

Gardena melinarthrum Dohrn.

Gardena melinathrum DOHRN, Emesina, Linn. Ent. 14 (1860) 214, 215 (Ceylon).

Gardena semperi DOHRN, Nachträge, Linn. Ent. 15 (1863) 64, 65 (Luzon).

Apparently *Gardena semperi* is the male of *G. melinarthrum*, as the only considerable character cited by Dohrn, "segments 1 and 2 of antenna strongly haired," is of sexual import only. The description of this species clears up a doubtful point in the original characterization; namely, as to relative lengths of the parts of the thorax. Here it is said that the "prothorax, not reckoning the part overlying the mesothorax," is as long as the mesothorax and metathorax together. The original description lacked the saving clause and was correspondingly misleading. Stål records *G. semperi* from the Philippines.[8]

With specimens in hand *Gardena bicolor* Distant [9] might prove separable from *G. melinarthrum*, but from the description it is not. Of the characters advanced, the length of the first antennal joint relative to the abdomen is sexual, and the degree of development of the wings is individual.

The genitalia of the oriental species of *Gardena* have not been described, so we append the following notes:

[8] Öfv. Vet.-Akad. Förh. 27 (1870) 704.

[9] Fauna British India, Rhynchota 2 (1904) 214, 215 (Burma).

Male.—Sixth sternite slightly and seventh very slightly emarginate, both medially and laterally; eighth sternite cuplike, opening upward, its hind margin with a median, erect, sharp spine; claspers slender, a little incurved, upturned and thickened apically; seventh tergite with a long, slightly spatulate, transversely corrugated flap, which extends nearly to apex of hypopygium (Plate 4, fig. 37).

Female.—Hind margin of seventh tergite nearly straight across; eighth tergite semielliptical, evenly convex posteriorly, ninth one and a half times as long as eighth, bluntly rounded apically, with a slight subapical, transverse elevation. Seventh sternite slightly concave laterally, and convex medially.

The genitalia in both sexes strongly resemble those of species of *Emesaya*, a different type from those of American species of *Gardena*.

Data for the specimens of *G. melinarthrum* examined in connection with the present paper are: Surigao, Iligan, Dapitan, Butuan, Mindanao; Mount Maquiling, Los Baños, Luzon (*Baker*). Samar, June 9, 1924 (*R. C. McGregor*). Kuala Lumpur, F. M. S., November 21, 1914; April 6, 1923 (*Pendlebury*).

The new variety *femoralis* is characterized by rather more copious, pale golden, sericeous pubescence throughout, and by the distinct, pale yellow, preapical annulus on fore femur. It does not differ appreciably in genital or other structural characters. Length, 25 millimeters.

Holotype, male, Kuala Tahan, Pahang, F. M. S., November 25, 1921 (*Pendlebury*).

Gardena brevicollis Stål.

Gardena brevicollis Stål, Hemiptera insularum Philippinarum, Öfv. Kgl. Vet.-Akad. Förh. 27 (1870) 701.

In general color the single specimen of this species we have examined is paler than typical examples of *G. melinarthrum;* the mid and hind legs are testaceous, with dark annuli on each side of the pale femorotibial joints. The small size, relatively shorter head and pronotum, and the armature of fore femur as described in the key are characteristic.

Length, 14 millimeters.

Kuala Lumpur, F. M. S., Gombak Valley, at light, October 13, 1921 (*Pendlebury*).

Genus BAGAUDA Bergroth

Most nearly related to *Ploiaria*, having the same type of wing and venation (Plate 4, figs. 38, 39). The fore legs are very similar to those of the subgenus *Luteva*, but the basal segment of tarsus occupies a greater proportion of the total length in *Bagauda*, there is no isolated anterior bristle near the base as in *Luteva*, and there is a distinct series of minute bristles between the antero- and posteroventral series in both species we have before us. For other characters see key.

Key to the species of Bagauda Bergroth.

1. Seventh sternite of female nearly transverse apically; anterior lobe of pronotum with an impressed median line ending in a well-defined pit just in front of constriction; vein emanating from apex of discal cell about as long as the cell, the transverse vein joining it well beyond its middle (Plate 4, fig. 38); all femora brownish.

B. brunneus sp. nov.

Seventh sternite of female distinctly produced in an apically rounded triangular flap; anterior lobe of pronotum with the median line scarcely impressed; vein emanating from apex of discal cell not over half as long as the cell, the transverse vein joining it before its middle (Plate 4, fig. 39); fore femur with a broad preapical pale annulus and mid and hind legs with the femorotibial joints whitish.

B. lucifugus sp. nov.

Bagauda lucifugus sp. nov.

Male and female.—Resembles *B. avidus* Bergroth in some respects, but is somewhat larger, has the wings shorter than the abdomen, and the fore femur with a broad preapical whitish band. More important characters are described in the key. Apical sternite of male with a deep V-shaped incision in middle of hind margin; margin of hypopygium without a spine. Venation of forewing as in Plate 4, fig. 39.

Length, 14 to 15 millimeters.

Holotype, female, allotype, 1 male and 3 female paratypes, Kuala Lumpur, F. M. S., Batu Caves, September 9 and 15, 1921, and May 6, 1923 (*Pendlebury*).

The insects of the genus *Bagauda* seem to have a liking for caves; two cavernicolous species have previously been described; namely, *B. tenebricola* Horvath [10] and *B. cavernicola* Paiva (Assam),[11] from both of which the present species is evidently distinct.

[10] Bull. Mus. d'Hist. Nat. Paris 16 (1910) 271 (East Africa).
[11] Rec. Ind. Mus. 16 (1919) 350–377, pls. 34–36.

Bagauda brunneus sp. nov.

Female.—Smaller than *B. lucifugus*, differing as stated in key and as follows: Mid and hind tibiæ narrowly pale at base, but the remainder of femorotibial articulation brownish. Venation of forewing as in Plate 4, fig. 38. Length, 9.5 millimeters (*B. avidus* is 14 to 15 millimeters).

MINDANAO, Lanao, Kolambugan, holotype (*Baker*).

Genus PLOIARIA Scopoli

Key to the subgenera and species of Ploiaria Scopoli.

1. Transverse impression of head with its extremities at about middle of eyes but curved backward so that its median part lies nearly as far posteriorly as any part of eyes (Plate 4, fig. 41); apparent posterior margin of prosternum as seen from below entire and convex posteriorly; discal cell of forewing four times as long as apical vein (Plate 4, fig. 40). *Megaploiaria* subg. nov.
   Subgenotype, P. (M.) fusca sp. nov.

   Transverse impression of head otherwise; apparent posterior margin of prosternum as seen from below more or less depressed and appearing emarginate in middle; discal cell of forewing shorter relative to apical vein ..... 2.
2. Transverse impression of head with its extremities near anterior margins of eyes, slightly convex posteriorly; posterior lobe of head with a stout bilobate dorsal tubercle; pronotum with two laterally projecting short tubercles just behind head, and two less-pronounced elevations just in front of median constriction, hind margin bisinuate; mesonotum with two more-prominent conical tubercles near posterior margin of disk; scutellum (apparently also part of the mesonotum) and basal abdominal tergite each with a long slender spine (Plate 3, fig. 36). *Gnomocoris* subg. nov.
   Subgenotype, P. (G.) spinosa sp. nov.

   Transverse impression of head almost straight across between middle of eyes; head, thorax, and base of abdomen without tubercles or spines ..... 3.
3. Fore trochanters each with at least one rather strong spine or bristle; combined length of fore tibia and tarsus (in the present species) fully as great as that of fore femur. Subgenus *Ploiaria* Scopoli.... 4.

   Fore trochanters without bristles, at most with some very short fine hairs; combined length of fore tibia and tarsus distinctly less than that of fore femur. Subgenus *Luteva* Dohrn ..... 5.
4. Length of discal cell of forewing subequal to that of the vein emitted from apex of the cell (Plate 4, fig. 44); apices of hind femora and bases of hind tibia broadly white..... P. (P.) subaequalis sp. nov.

   Length of discal cell of forewing about twice as great as that of vein emitted from apex of the cell (Plate 4, fig. 45); hind femora and tibiæ uniformly brownish..... P. (P.) uniformis sp. nov.

5. Some, usually dark, transverse veinlets in the cell proximad of the discal cell (Plate 4, fig. 46); mid and hind femora without an outstanding preapical dark band ..... 6.
No transverse veinlets in the cell proximad of the discal cell; width of eye much less than interocular space; mid and hind femora each with a conspicuous broad fuscous preapical band.
P. (L.) mellea sp. nov.
6. Males ..... 7.
Females ..... 10.
7. Apex of hypopygium with a distinct central spine ..... 9.
Apex of hypopygium without a central spine ..... 8.
8. (Four alternatives.) Apex of hypopygium merely angulate, the apex recurved and slightly emarginate (Plate 4, fig. 48).
P. (L.) apicata sp. nov.
Apex of hypopygium nearly straight across (Plate 4, fig. 49).
P. (L.) recta sp. nov.
Apex of hypopygium slightly emarginate (Plate 4, fig. 51); stigma rufous beyond cross vein ..... **P. (L.) media sp. nov.**
Apex of hypopygium deeply emarginate (Plate 4, fig. 54); stigma not rufous ..... **P. (L.) ultima sp. nov.**
9. Spine of hypopygium set distinctly within posterior margin (Plate 4, fig. 50); fuscous species, with apices of mid and hind femora darker.
P. (L.) bakeri sp. nov.
Spine of hypopygium a continuation of posterior surface (Plate 4, fig. 53); testaceous species, with apices of mid and hind femora paler.
P. (L.) nitida sp. nov.
10. Transverse thickening of membrane of hind wing narrow (Plate 4, fig. 52) ..... 11.
Transverse thickening of membrane of hind wing broad (Plate 4, fig. 47) ..... 12.
11. Pronotum highly glossy, the fine pubescence difficult to see; mesonotum with fine barring laterally ..... **P. (L.) zebrina sp. nov.**
Pronotum subshining, the fine pubescence obvious; mesonotum without barring ..... **P. (L.) ultima sp. nov.**
12. Pronotum highly glossy, the fine pubescence difficult to see.
P. (L.) apicata sp. nov.
Pronotum subshining, the pubescence obvious.... **P. (L.) nitida sp. nov.**

Ploiaria (Megaploiaria) fusca sp. nov.

Male.—General color blackish brown, bases of antennæ, tylus, two short lateral and one longer median vitta on anterior lobe of head (Plate 4, fig. 41), marmorations, mostly median, on pronotum, three narrow vittæ on disk and margins of mesonotum, costa basally, spiracles, marmorations on venter, mid and hind trochanters, bases and apices, and indistinct annuli on shaft of femora, and basal annuli on tibiæ yellowish. Forewing fuscous, without pale markings.

Basal segment of antenna with moderately long erect hairs, tylus evident from side view, longer from antennal insertion

than head is from that point to eye; seventh tergite convex apically; sixth sternite almost straight in middle, more or less emarginate laterally; wings a fourth shorter than abdomen; transverse thickening of hind wing very broad, nearly straight-sided, and blackish; apex of hypopygium with a broad process which is shallowly angularly emarginate in center (Plate 4, fig. 42); claspers terete, incurved and acute apically. Venation of forewing as in Plate 4, fig. 40.

Length, 19 millimeters.

MINDANAO, Surigao, Surigao, holotype (*Baker*).

The fore legs are missing in the specimen described, and we may err in assigning it to *Ploiaria*, with which it agrees however in general structure of thorax, in venation, and in the possession of the transverse thickening in the hind wing.

Ploiaria (Gnomocoris) spinosa sp. nov.

Female.—Dark brown, shiny, pronotum variegated with pale yellowish; mesonotum with four round spots along each margin and some on disk, yellowish, upper margin of pleura stramineous. Antenna dark brown, basal two segments with many narrow pale annuli. Fore legs dark brown, variegated with yellowish, more prominently so at middle, femora each with two irregular annuli, one just beyond middle, the other at apex, tibiæ and tarsi each with three pale annuli, one at base, one at middle, and the other at tip, the latter not complete on tibiæ; mid and hind legs testaceous, copiously dotted and subannulate with pale brown, a narrow preapical annulus and a broader apical band on each femur, and a narrow stripe near base of each tibia, blackish. Wings brown, with series of rounded to subquadrate hyaline spots in each cell, close to veins and separated by brown lines (Plate 4, fig. 43).

Head, thorax, and fore legs as in Plate 3, fig. 36; note the snoutlike prolongation of head in front of antennal insertions, the corrugate fore coxa, the undulate fore femur, a few basal spines of the armature much stronger than the others, and the trisinuate posterior margin of pronotum. Antennæ with only minute appressed hairs, third and fourth segments subequal. A laterally projecting tubercle each side of apex of tylus. Constriction of pronotum more pronounced than usual in the genus, and farther removed from posterior end of the segment; mesonotum sulcate in center. Venation of apical part of forewing as in Plate 4, fig. 43; transverse thickening of hind wing broad and curved.

Length, 8 millimeters.

BORNEO, Sandakan, holotype (*Baker*).

Despite its unusual tubercles and spines, this species fits well in the large genus *Ploiaria*, agreeing in wing venation and texture, including the transverse thickening of hind wing, as well as in the fundamental structure of thorax and fore legs.

Ploiaria (Ploiaria) subaequalis sp. nov.

Male.—General color testaceous, antennæ and hind legs fuscous, the femorotibial joints ivory colored; forewings with the veins fuscous, and sparse, transverse dusky irrorations (Plate 4, fig. 44), which appear in the iridescent membrane like watermarks in silk.

Antenna with soft hairs, more or less erect basally, but appressed apically; fore femora with mixed long and short spines, the former curved, but neither so strongly curved nor so contrasted in size with the shorter spines as is the case in some of the American species; fore trochanter slightly pointed-tuberculate beneath, with one long and one short spine; mesonotum with a median impressed line; transverse thickening of hind wing rather broad and curved; venation of forewing as in Plate 4, fig. 44; hind margin of hypopygium with two slender, erect, acute spines; claspers lanceolate, incurved, and acute apically.

Length, 6 millimeters.

LUZON, Laguna, Los Baños, holotype (*Baker*).

Ploiaria (Ploiaria) uniformis sp. nov.

Female.—General color testaceous, eyes black, basal segment of antenna blackish, mid and hind legs brownish; forewings yellowish hyaline, the veins darker, an oblong dark spot in middle of discal cell, and in next distal cell along costa (Plate 4, fig. 45); apical cross vein overlaid by a narrow dusky marking; stigma reddish apically.

Basal segment of antenna not noticeably hairy; fore trochanter rounded beneath, with a single spine; armature of fore femur rather uniform, no outstanding spines; pronotum and mesonotum each with a median impressed line; mid and hind femora slightly clavate. Apical venation of forewing as in Plate 4, fig. 45.

Length, 6.5 millimeters.

MINDANAO, Surigao, Surigao, holotype (*Baker*).

Examination of such species as this and the preceding shows that there is no real dividing line between the groups *Ploiaria*

and *Luteva*. All gradations in the trochanteral and femoral armature exist; and in other characters the groups agree so closely that they appear best placed in a single comprehensive genus.

Ploiaria (Luteva) mellea sp. nov.

Female.—Body dark, antennæ and legs pale honey color; forewings stramineous, dusky along margins and veins, hyaline in cells. First and second segments of antenna with a fuscous subapical and a whitish apical annulus, third segment dusky, with pale apex, fourth entirely dusky. Mid and hind femora with a subapical and tibiæ with a subbasal brownish band or spot, in each case there is a smaller spot nearer the articulation; segments of fore legs more or less brownish beneath, the markings tending to form three half annuli on femur, and to cover the entire apex of tibia.

Body, antenna, and legs with abundant short, pale pubescence, rather erect on pronotum and mesonotum; each of these divisions with a median impressed line.

Length, 12 millimeters.

LUZON, Laguna, Mount Maquiling, holotype female, and paratype, without abdomen (*Baker*).

Ploiaria (Luteva) apicata sp. nov.

Male and female.—Body, antennæ, and legs stramineous to testaceous, wings whitish to yellowish hyaline. First segment of antenna with a whitish subapical annulus, second narrowly whitish at base and apex. Fore coxa and tibia more or less infuscated, femur with three more or less distinct fuscous annuli. Mid and hind femora each with subapical whitish annulus, the femorotibial joint broadly whitish, with subarticular brownish spots or narrow annuli. Veins of forewing darker, transverse ones with narrow dusky clouding (Plate 4, fig. 46).

Antenna of male with long spreading hairs basally, decreasing in length gradually to apex of second segment; third and fourth segments with fine appressed pubescence. Mesonotum with a median impressed line. Thickened part of hind wing as in Plate 4, fig. 47. Apex of hypopygium as described in key (Plate 4, fig. 48); claspers terete, incurved, acute.

Length, 10.5 to 12.5 millimeters.

BORNEO, Sandakan, holotype male, allotype female, and 15 paratypes (*Baker*).

Ploiaria (Luteva) recta sp. nov.

Male.—General color stramineous, tinged with reddish, especially on fore legs, where it tends to form annuli on femora. Antenna and hind femur without pale annuli. Forewing whitish hyaline, with a vein from base to near middle, and apical part of stigma, reddish; other veins and cross veinlets fuscous, narrowly dusky margined, a dark dot between costa and apex of discal cell.

Antenna with long hairs basally, as usual in males of the group. Mesonotum with a longitudinal median impression. Hind margin of hypopygium straight between claspers, the latter lanceolate, incurved, and acute (Plate 4, fig. 49).

Length, about 11 millimeters.

MINDANAO, Surigao, Surigao, holotype (*Baker*).

Ploiaria (Luteva) media sp. nov.

Male.—Colored much like *P. recta*, but with both the reddish and the dusky markings fainter; apex of hypopygium as in Plate 4, fig. 51; claspers terete, incurved, acute.

Length, about 10 millimeters.

LUZON, Laguna, Mount Maquiling, holotype (*Baker*).

Ploiaria (Luteva) ultima sp. nov.

Male and female.—General color testaceous, mid and hind femorotibial joints whitish, with included spots or faint limiting annuli, dusky. Basal transverse veinlets and veins of forewing dusky, membrane yellowish hyaline.

Antenna of male with long spreading hairs basally, shorter and appressed ones apically. Thickened part of hind wing as in Plate 4, fig. 52. Apex of hypopygium as in Plate 4, fig. 54; claspers terete, incurved, and acute.

Length, 10 to 13 millimeters.

Holotype male, allotype, and 18 paratypes, Mount Maquiling, Luzon; paratypes, Malinao, Tayabas; Los Baños, Laguna; Cuernos Mountains, Negros (*Baker*).

Ploiaria (Luteva) bakeri sp. nov.

Male.—Head fuscous above, piceous below; thorax castaneous above, piceous below; abdomen piceous. Basal two segments of antenna with testaceous and dusky annuli, darkest near extremities; apical two segments fuscous. Beak banded. Fore coxa piceous, with some yellow markings on dorsal surface near apex; trochanter piceous; femur piceous beneath, marbled with

fuscous on basal half of upper surface, and with a subapical fuscous annulus; tibia piceous, with subbasal pale annulus; tarsus piceous, pale at each extremity. Mid and hind femora castaneous, slightly mottled with yellowish, each with a subapical annulus of the same color; tibiæ of these legs testaceous, each with a pale annulus bordered by two fuscous ones near base. Dusky transverse markings of forewing distinct and occurring almost throughout the whole length of the wing; a distinct piceous spot near base of discal cell.

Antenna haired as usual in males of the genus. Hypopygium as in Plate 4, fig. 50.

Length, 10.5 millimeters.

MINDANAO, Surigao, Surigao, holotype (*Baker*).

Ploiaria (Luteva) nitida sp. nov.

Male and female.—General color stramineous to testaceous, antennæ dusky apically, head brownish, glossy; pronotum highly polished, mesonotum somewhat duller; femorotibial joints of mid and hind legs whitish, the segments reddish tinged near by, giving the effect of a subapical pale annulus on femur. Forewing hyaline, most of the longitudinal veins yellowish, the transverse veins and veinlets dusky, and a dusky spot or vitta in inner cell near apex.

Hypopygium as in Plate 4, fig. 53.

Length, 11 to 13 millimeters.

Holotype male, allotype, and 17 paratypes, Surigao, Mindanao; paratypes, Butuan; Davao, Iligan, Mindanao; Basilan (*Baker*).

Ploiaria (Luteva) zebrina sp. nov.

Female.—General color testaceous, the legs paler, the head, thorax beneath, and abdomen darker; mid and hind femorotibial joints whitish. Costal and radial margins of forewing yellowish, the veins otherwise fuscous, margined with dusky, transverse veinlets dusky.

Length, 12 millimeters.

NORTHWESTERN PANAY, holotype (*Baker*).

Genus ISCHNONYCTES Stål

Ischnonyctes alatus Distant.

Ischnonyctes alatus DISTANT, Fauna Brit. Ind. Rhynchota 2 (1904) 217, fig. 153 (Ceylon).

One male specimen from Mount Maquiling, Luzon (*Baker*), answers to Distant's description, so we are using his name provisionally. The genitalia agree with those of *I. marcidus* Uhler,[12] of which form *I. alatus* may be only the winged male.

Genus ISCHNOBAENA Stål

Ischnobaena macerrima Stål.

Ischnobaena macerrima STÅL, Öfv. Kgl. Vet.-Akad. Förh. 27 (1870) 703 (Philippines).

Ischnobaena dohrnii STÅL, op. cit. pp. 703, 704 (Philippines).

We identify as this species a specimen from Mount Maquiling, Luzon (*Baker*); its characters differ from those mentioned in the original description as follows: Less contrasted in coloration, the general color being castaneous, the fore tibia with a broad pale annulus at middle, the mid and hind tibiæ with two indistinct pale annuli on basal third, and each of the tergites except first with a pale blotch near each anterior angle. Such pale markings are not likely to be very constant and, since differences in them were the only distinctions pointed out in Stål's original descriptions, his two names may apply to a single species. In the specimen before us, a female, the last tergite is triangular, with the sides declivate, forming a lidlike structure; the sternites from 2 to 5 are emarginate medianly, least so on 5, and they are traversed by a median line which upon 6 is elevated and prolonged upon a moderate triangular median process.

Length, 34 millimeters.

Genus PHRYXOBOTRYS novum

In the structure of the fore leg (Plate 4, fig. 55) this genus is most like the subgenus *Ploeodonyx* in *Ghilianella*. However, it differs from that group in having the fore tarsal claw half as long as, and movably articulated with, the tarsus, and diverges from *Ghilianella* in general in the lack of denticles on underside of fore tarsus (which is merely serrulate and provided with two rows of setulæ) and fore tibia (which is long setose), in the lack of a frontal prominence or spine, and in the polished character of its whole surface, there being no granulations as frequently is the case in *Ghilianella*. The armature of the fore femur (Plate 4, fig. 55) is highly characteristic, being confined to somewhat less than the distal half of the undersurface, and consisting in the main of a single row of sharp spines, which

[12] *Emesa marcida* Uhler, Proc. U. S. Nat. Mus. 19 (1896) 273 (Japan).

are situated on almost the median ventral surface; there is a long spine close to the base of this series (and near apex of opposed tarsus) which is situated on the anteroventral surface, and basad of this a series of three long spines which runs obliquely across the vental surface, directed obliquely forward over tarsal claw.

Notwithstanding these differences and the notably small size (less than half the length of any described species), it is not improbable that intergrading forms may be found that will make it desirable to regard this segregate as a subgenus of *Ghilianella*.

Genotype, the following new species:

Phryxobotrys castanea sp. nov.

General color of head and body dark castaneous, with a pale spot on middle of hind margin of mesonotum and of metanotum; first tergite pale anteriorly, and a pale spot on connexivum of each abdominal segment. Fore leg pale castaneous, the articulations paler; mid and hind legs testaceous, with a faint dark annulus each side of the pale femorotibial joint. Antenna testaceous basally, darker apically.

Entire surface of head and body highly polished. Mesonotum and metanotum each with margins and a median carina moderately roundedly elevated; their posterior angles rounded. Abdomen clavate, attaining its greatest width at about the juncture of segments 3 and 4 and narrowing very little posteriorly; eighth tergite narrowly semielliptical, ninth truncate triangular, rounded apically; seventh sternite somewhat produced and rounded medianly, slightly emarginate laterally. Fore coxa nearly as long as fore femur.

Length, 6 millimeters.

LUZON, Laguna, Mount Maquiling, holotype female (*Baker*).

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ILLUSTRATIONS

PLATE 1

FIG. 1. *Emesopsis* (*Hadrocranella*) *neptunis* sp. nov., forewing.
2. *Emesopsis* (*Hadrocranella*) *obsoletus* sp. nov., forewing.
3. *Emesopsis* (*Emesopsis*) *gaius* sp. nov., forewing.
4. *Emesopsis* (*Pseudobolos*) *emmesius* sp. nov., forewing.
5. *Emesopsis* (*Emesopsis*) *nero* sp. nov., discal cell of forewing.
6. *Emesopsis* (*Emesopsis*) *gaius* sp. nov., discal cell of forewing.
7. *Emesopsis* (*Pseudobolos*) *connexus* sp. nov., base of radius and adjoining veins of forewing.
8. *Emesopsis* (*Pseudobolos*) *emmesius* sp. nov., base of radius and adjoining veins of forewing.
9. *Emesopsis* (*Pseudobolos*) *velutinervis* sp. nov., base of radius and adjoining veins of forewing.
10. *Emesopsis* (*Hadrocranella*) *neptunis* sp. nov., fore coxa and femur, armature omitted.
11. *Emesopsis* (*Hadrocranella*) *obsoletus* sp. nov., fore coxa and femur, armature omitted.
12. *Emesopsis* (*Emesopsis*) *spicatus* sp. nov., fore coxa and femur, hairs and markings omitted.
13. *Emesopsis* (*Emesopsis*) *spicatus* sp. nov., apex of male hypopygium from side.
14. *Emesopsis* (*Emesopsis*) *spicatus* sp. nov., apex of male hypopygium from behind.
15. *Emesopsis* (*Emesopsis*) *nero* sp. nov., apical process of hypopygium from behind.
16. *Emesopsis* (*Pseudobolos*) *moniliferus* sp. nov., hypopygium of male from side.

PLATE 2

FIG. 17. *Emesopsis* (*Hadrocranella*) *neptunis* sp. nov., hypopygium of male from side; *a*, apex of apical tergite.
18. *Emesopsis* (*Emesopsis*) *spicatus* sp. nov., hypopygium of male from side.
19. *Emesopsis* (*Emesopsis*) *gaius* sp. nov., hypopygium of male from side.
20. *Emesopsis* (*Emesopsis*) *gallienus* sp. nov., hypopygium of male from side.
21. *Emesopsis* (*Emesopsis*) *nero* sp. nov., hypopygium of male from side.
22. *Emesopsis* (*Emesopsis*) *hadrian* sp. nov., hypopygium of male from side.

PLATE 3

FIG. 23. *Ademula reticulata* sp. nov., forewing.
24. *Ademula reticulata* var. *abluta* var. nov., apex of male hypopygium from behind.
25. *Ademula nubecula* sp. nov., apex of male hypopygium from behind.
26. *Tridemula plurima* sp. nov., forewing, markings omitted.
27. *Tridemula plurima* sp. nov., apex of male hypopygium from behind.
28. *Tridemula pallida* sp. nov., apex of male hypopygium from behind.
29. *Tridemula plurima* sp. nov., ventral view of thorax and base of abdomen.
30. *Empicoris bilineatus* sp. nov., discal and adjoining cells of forewing.
31. *Empicoris bakeri* sp. nov., apex of male abdomen from below.
32. *Empicoris discalis* sp. nov., apex of male abdomen from below.
33. *Stenolemus plumosus* Stål, apex of hind femur and base of hind tibia.
34. *Myiophanes fluitaria* sp. nov., forewing.
35. *Myiophanes annulifera* sp. nov., forewing.
36. *Ploiaria* (*Gnomocoris*) *spinosa*, head, thorax, and base of abdomer from side.

PLATE 4

FIG. 37. *Gardena melinarthrum* Dohrn, hypopygium of male from side.
38. *Bagauda brunneus* sp. nov., forewing.
39. *Bagauda lucifugus* sp. nov., forewing.
40. *Ploiaria* (*Megaploiaria*) *fusca* sp. nov., apical part of forewing.
41. *Ploiaria* (*Megaploiaria*) *fusca* sp. nov., head from above.
42. *Ploiaria* (*Megaploiaria*) *fusca* sp. nov., apex of male hypopygium.
43. *Ploiaria* (*Gnomocoris*) *spinosa* sp. nov., apical part of forewing.
44. *Ploiaria* (*Ploiaria*) *subaequalis* sp. nov., apical part of forewing.
45. *Ploiaria* (*Ploiaria*) *uniformis* sp. nov., apical part of forewing.
46. *Ploiaria* (*Luteva*) *apicata* sp. nov., forewing.
47. *Ploiaria* (*Luteva*) *apicata* sp. nov., thickened part of hind wing.
48. *Ploiaria* (*Luteva*) *apicata* sp. nov., apex of male hypopygium.
49. *Ploiaria* (*Luteva*) *recta* sp. nov., apex of male hypopygium.
50. *Ploiaria* (*Luteva*) *bakeri* sp. nov., apex of male hypopygium.
51. *Ploiaria* (*Luteva*) *media* sp. nov., apex of male hypopygium.
52. *Ploiaria* (*Luteva*) *ultima* sp. nov., thickened part of hind wing.
53. *Ploiaria* (*Luteva*) *nitida* sp. nov., apex of male hypopygium.
54. *Ploiaria* (*Luteva*) *ultima* sp. nov., apex of male hypopygium.
55. *Phryxobotrys castanea* sp. nov., fore leg.

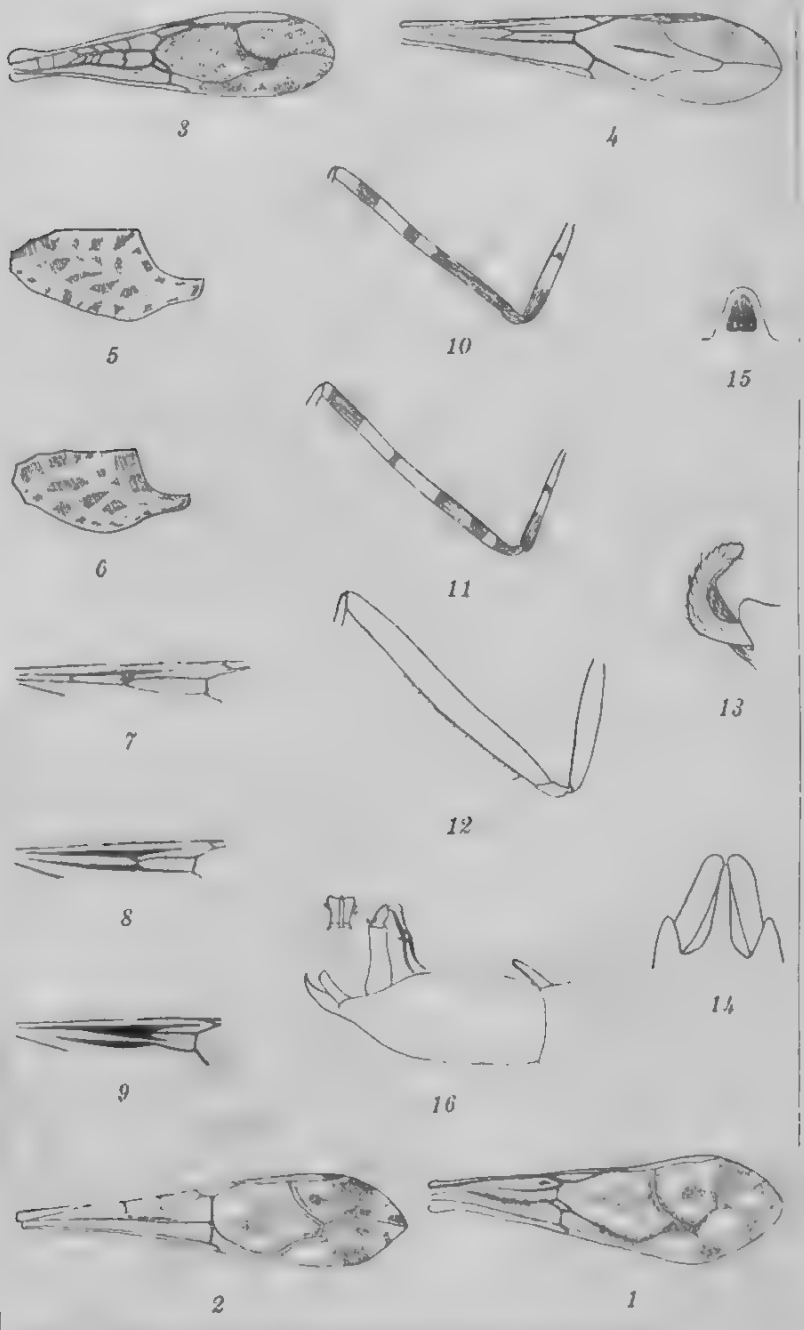
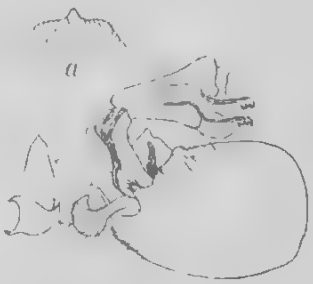


PLATE 1.



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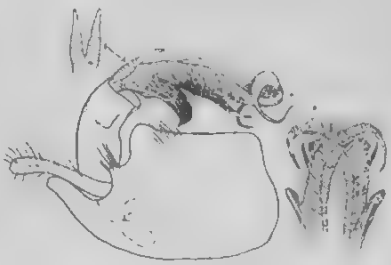

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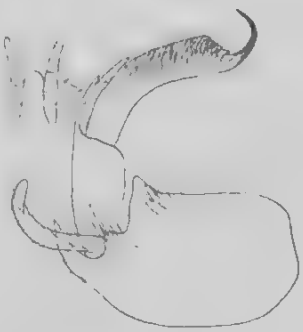
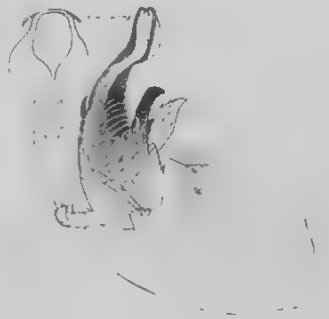


18

21



19

22

PLATE 2.

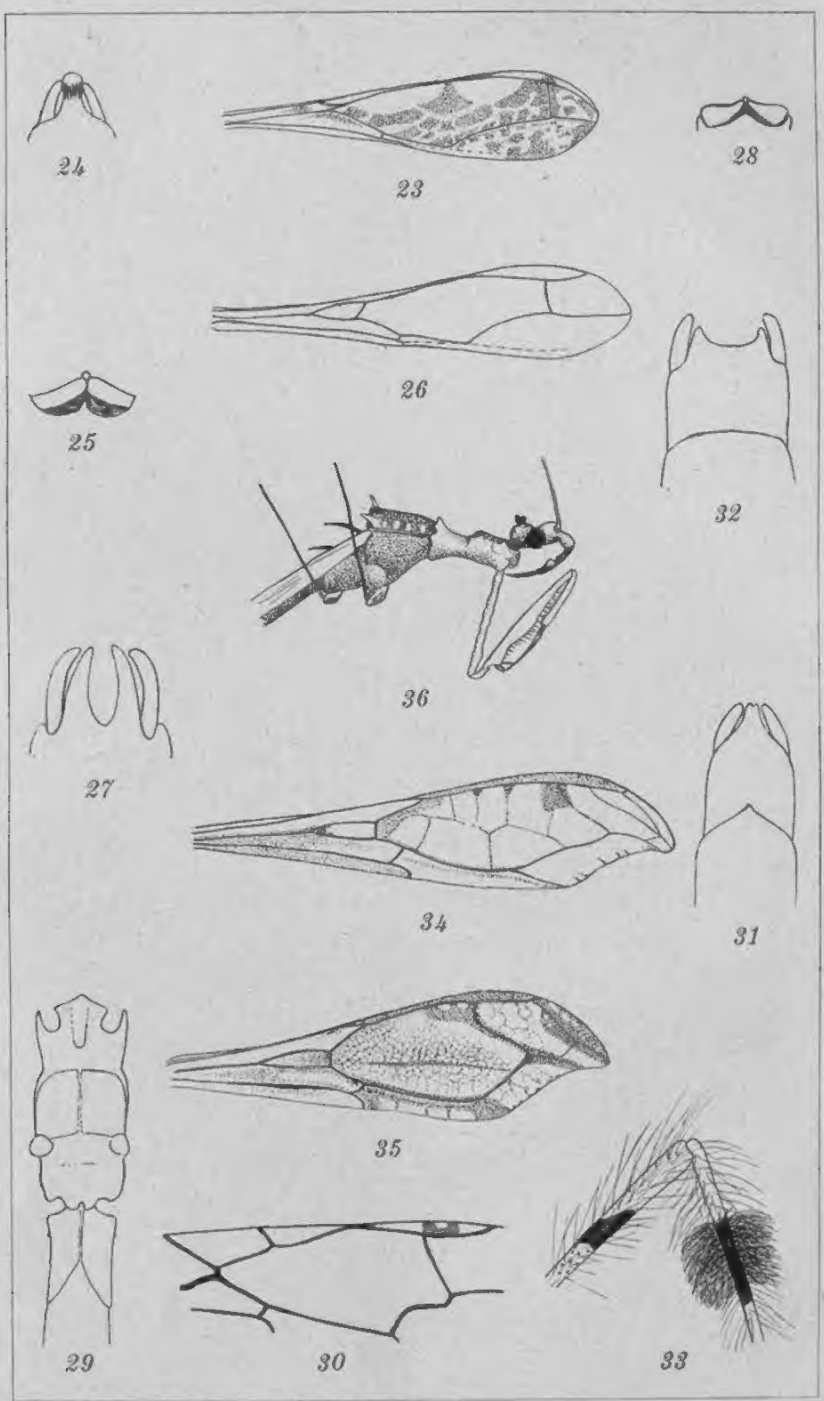


PLATE 3.

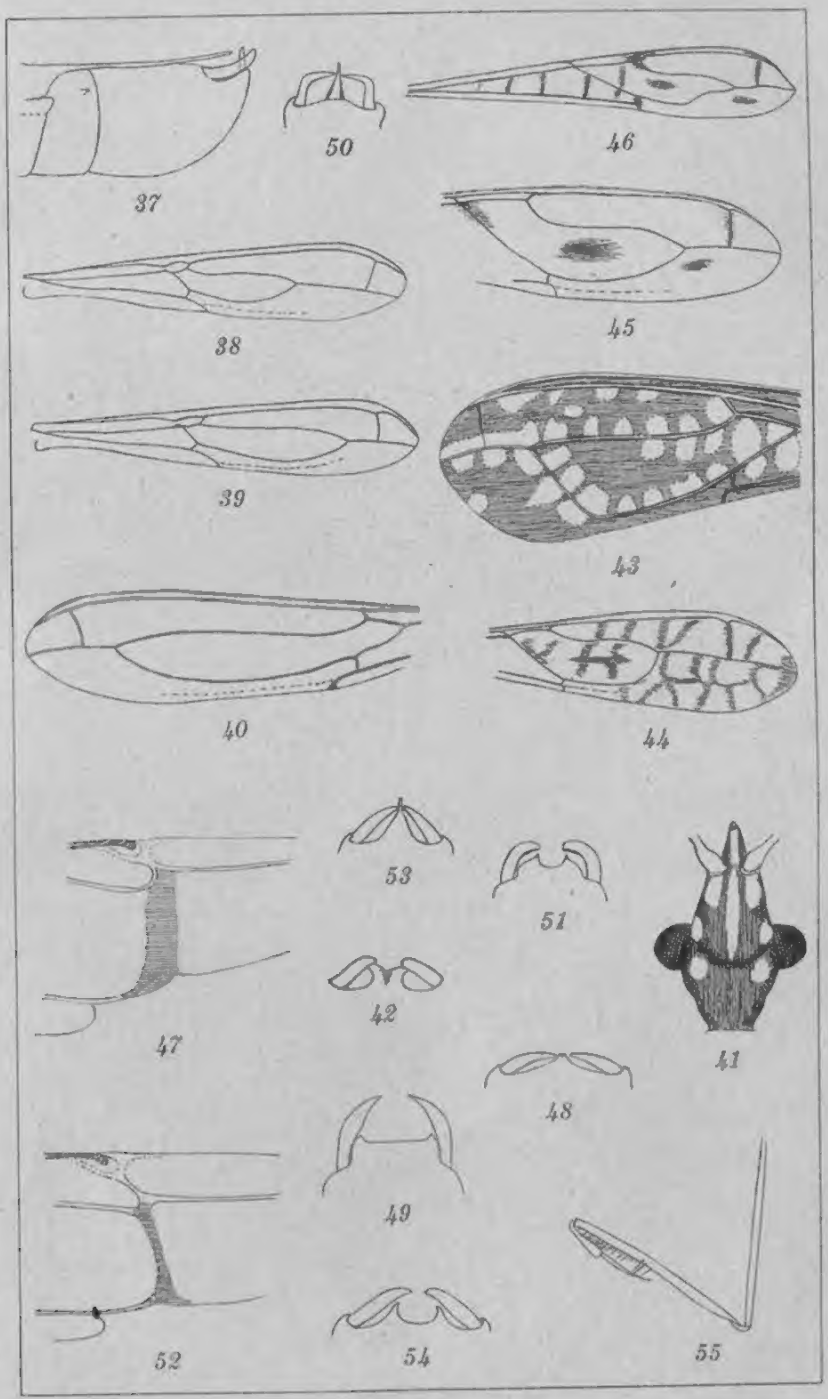


PLATE 4.

CONTENTS

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